CANNABINOID conference 2013

International Association for Cannabinoid Medicines

IACM 7th Conference on Cannabinoids in Medicine

27-28 September 2013
Cologne, Germany

PROGRAM & ABSTRACTS

cannabinoid medicines International Association for Cannabinoid Medicines

Place

Leonardo Royal Hotel Köln am Stadtwald, Dürener Strasse 287, 50935 Cologne, Germany

Registration Fee

250 Euros for both days with a registration until 31 July 2013
(300 Euros after 31 July 2013)
Students pay a reduced fee of 150 Euros for both days with a registration until 31 July 2013
(200 Euros after 31 July 2013)
The registration fees include a copy of the abstract book, daily rates (lunch for both days, coffee during the breaks) and the evening dinners on Friday and Saturday.

Organizer

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www.iacm2013.org

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We gratefully acknowledge sponsoring by:

GENERAL INFORMATION

Badges

Please wear your badge at all times during the conference. You will also need to wear it for the evening dinners in order to you receive your drink coupons.

Conference Dinners

On Friday evening we will have finger food to allow better social networking.
The evening dinner on Saturday will start at about 20:00 h and will be a generous buffet with beef, poutry, fish, salads, different desserts, etc.

All Conference Dinners will be at the conference place: Leonardo Royal Hotel Köln Am Stadtwald.

The food as well as the first two drinks at the evening dinner on Saturday are covered by your registration fee. Please pay for additional drinks on Saturday and the drinks on Friday. Coupons will be provided at the hotel, so please wear your badge for dinner.

Poster Sessions

There will be two poster sessions at the conference:
Session 1: Friday, September 27, 13:00 - 15:00 during and after the lunch break
Session 2: Saturday, September 28, 13:30 - 15:30 during and after the lunch break

All posters will be available until the end of Poster Session 2 on Saturday. Presenters of posters with even numbers will be present at Poster Session 1, presenters of posters with odd numbers will be present at Poster Session 2.

Friday, September 27

08:15 - 17:00		**Registration**
	Chair	Presentation
09:00 - 09:40	Ethan Russo	**Plenary Lecture 1** Philip Robson: "Cannabinoids and mental health: benefits and risks"
09:40 – 09:55 09:55 – 10:10 10:10 – 10:40 10:40 – 11:00	William Notcutt	**Session I – The needs of the patient** Sarah Martin: "Amazing Multiple Sclerosis Recovery Using Sativex Tina Silva: "The potential of cannabis when consumed in its natural form" Arno Hazekamp: "Studies, surveys and statistics - do we know what patients want?" Discussion
11:00 – 11:15		Break
11:15 – 11:45 11:45 – 12:15 12:15 – 12:45 12:45 – 13:00	Donald Abrams	**Session II – The needs of the clinician** Mark Ware: "The educational needs of physicians regarding cannabis and cannabinoids" Willy Notcutt: "The Clinical Management of the Patient using Cannabinoids" John Zajicek: "Where are we with the use of cannabinoids in multiple sclerosis?" Discussion
13:00 – 15:00		Lunch and **poster session 1**
15:00 – 15:30 15:30 – 16:00	Mark Ware	**Session III – The needs of the pharmaceutical industry** Ethan Russo: "The Sativex Solution to the Medicinal Cannabis Challenge" Tjalling Erkelens: "Herbal cannabis: the way it fits in to today's pharmaceutical practice."

16:00 – 16:15 16:15 – 16:25		Luigi Romano: "Cannabis oil: chemical evaluation of an upcoming cannabis-based medicine" Discussion
16:25 - 16:40		Break
16:40 – 18:00		**IACM General Meeting**
19:00 – 22:00		**Social and networking with buffet**

Saturday, September 28

08:15 - 12:00		**Registration**
	Chair	Presentation
09:00 – 09:40	Raphael Mechoulam	**Plenary Lecture 2** Roger Pertwee: "The pharmacology and therapeutic potential of plant cannabinoids: great expectations"
09:40 – 10:10 10:10 – 10:40 10:40 – 10:50	Daniela Parolaro	**Session IV - The needs of the scientist** Vincenzo Di Marzo: "The need for mechanisms: how much do the phytocannabinoid and endocannabinoid worlds overlap?" Tibor Harkany: "The molecular basis of cannabis sensitivity in the developing brain" Discussion
10:50 – 11:05		Break
11:05 – 11:35 11:35 – 12:05	Vincenzo Di Marzo	**Session V - Preclinical/translational studies** Javier Fernandez Ruiz: "CB_2 receptors as a promising target for developing disease-modifying therapies in neurodegenerative disorders" Daniela Parolaro: "Phytocannabinoids: a new

12:05 – 12:35 12:35 – 12:55 12:55 – 13:10 13:10 – 13:30		opportunity for psychosis (preclinical evidence)" Manuel Guzman: "Phytocannabinoids as potential anticancer agents" Itai Bab: "Major Cannabis Constituents Stimulate Fracture Healing" Yosef Sarne: "Multi-protective effects of tetrahydrocannabinol (thc): ultra-low doses defend the brain, the heart and the liver" Discussion
13:30 – 15:30		Lunch and **poster session 2**
15:30 – 16:00 16:00 – 16:15 16:15 – 16:30	Willy Notcutt	**Session VI – Clinical studies** Donald Abrams: "Cannabinoids in Pain and Palliative Care: An Update" Jeffrey C. Raber: "Cannabinoid and terpenoid profiling of medicinal cannabis in California" Discussion
16:30 – 16:45		Break
16:45 – 17:15	Franjo Grotenhermen	**Open forum and IACM member input: where are we going and how do we get there?**
17:15 – 18:00	Roger Pertwee	**Special Presentation** Raphael Mechoulam: „The Road Ahead for Cannabis Research"
20:00 – 22:30		**Dinner with Award Ceremony**

Please note in your diary:

The **IACM 8th Conference on Cannabinoids in Medicine**
together with the European Workshop on Cannabinoids
is anticipated for 17-19 September 2015 in Sestri Levante, **Italy**.

Posters

Poster Sessions:

Friday, 13:00 – 15:00

Saturday, 13:30 – 15:30

All posters will be available until the end of Poster Session 2 on Saturday. Presenters of posters with even numbers will be present at Poster Session 1, presenters of posters with odd numbers will be present at Poster Session 2.

1	Moshe Geitzen, Zach Klein, Lihi Bar-Lev Schleider, RNA Inbal Sikorin	Cannabis in the Third Age –Experimental Therapy at a Nursing Home
2	Ramy Ammar, Mona El-Azab, Yasser Moustafa and Gudrun Ulrich-Merzenich	The Antitumor Potential of the Endocannabinoid Reuptake Inhibitor OMDM-2 and its Combination with Curcumin
3	Janire Sáez, Oier Aizpurua, Jone Omar, Patricia Navarro, Aresatz Usobiaga Nestor Etxebarria.	A quantitative determination of cannabinoids in plasma and urine using gas chromathography-mass spectrometry.
4	Oier Aizpurua, Janire Saez, Jone Omar, Patricia Navarro, Nestor Etxebarria and Aresatz Usobiaga	Cannabinoid fingerprinting of 31 cannabis sativa L. varieties using high-performance liquid chromatography tandem mass spectrometry
5	István Ujváry	Δ^9-tetrahydrocannabinol-11-oic acid, a ubiquitous yet underresearched cannabinoid. An overview of the literature
6	Maria Grazia Cascio, Pietro Marini and Roger G. Pertwee	Effects of Δ^9-tetrahydrocannabivarin at serotoninergic 5-HT_{1A} receptors, and of its 11-hydroxy-metabolite at cannabinoid CB_1 and CB_2 receptors.
7	Alexander Fuchs, Viktor Rempel and Christa Müller	The natural product magnolol as a lead structure for the development of potent cannabinoid receptor agonists
8	Robert Günther, Rodrigo Teodoro, Steffen Fischer, Rareş-Petru Moldovan, Corinna Lueg, Winnie Deuther-Conrad, Bernhard Wünsch and Peter Brust	Radiolabelling and biodistribution studies of Potential Radioligands for PET-imaging of cannabinoid receptor type 2 (CB_2)
9	Javier Pedraza Valiente	Cannabis social clubs as a new source of observational data
10	Zlatko Mehmedic, Mohamed M. Radwan, Amira S. Wanas, Ikhlas Khan and Mahmoud A. ElSohly	Antifungal activity of the volatile oil of high potency cannabis sativa L. Against cryptococcus neoformans

11	Valerio Chiurchiù, Maria Teresa Cencioni, Elisa Bisicchia, Marco De Bardi, Claudio Gasperini, Giovanna Borsellino, Diego Centonze, Luca Battistini and Mauro Maccarrone	Distinct alterations of the endocannabinoid system in human myeloid and plasmacytoid dendritic cells during multiple sclerosis
13	Yuval Zolotov, Amotz Perelmann	Licensed medical cannabis patients report safe driving behavior
14	Sébastien Béguerie and Ignacio García	Comparative study for the quantification of THC, CBD and CBN between TLC alpha-CAT method and GC-FID
15	Thomas Baechler, Gordon Dobritzsch	Hemp meristem-macerates in gemmotherapy
16	Ilya Reznik	Neuropsychatric uses of medical cannabis/marijuana: focus on post-traumatic stress disorder
17	Paul Hornby and Adolfo Gonzalez	Reduction in methadone consumption and withdrawal symptoms with ingestion of standardized oral cannabis. An observational/feasibility study.
18	Christian Lanz, Johan Mattsson, Mehmet Ali Umut Soydaner and Rudolf Brenneisen	Medicinal cannabis: in vitro validation of vaporizers for the smoke-free inhalation of cannabinoids
19	Carmine Giorgio, Ignacio García, Jaime Carrion, Scott Blackey	Development of neutral-day cannabis plants with high CBD content
20	Martin F.J. Perescis, Annika Lüttjohann, Lyudmila Vinogradova, Gilles van Luijtelaar - Clementina M. van Rijn	Rats chronically treated with a cannabinoid antagonist: convulsive seizures and decreased synchronization in the EEG
21	Köhnemann S, Valverde L, Lischka C, de Meijer EP	A cannabis-DNA analysis method to secure the genetic identity of cannabis plants
22	Clementina M. van Rijn, Lyudmila V. Vinogradova.	Epileptogenesis under control of the endogenous cannabinoid system?
23	Angelo A. Izzo, Mohamed S Zaibi, Ed Wargent, Raffaele Capasso, Carolyn Arbuckle, Marnie Duncan, Vincenzo Di Marzo, Michael A. Cawthorne, Roger G. Pertwee	The effect of delta-9-tetrahydrocannabivarin on food deprivation-induced food intake and upper gastrointestinal motility: differences from rimonabant
24	Ben Wedeking, Ina Pinker and Gianpaolo Grassi	Plant regeneration of Cannabis sativa [L.], through anther culture (in vitro) of the cultivar “USO’ S”

Oral Presentations

CANNABINOIDS AND MENTAL HEALTH: RISKS AND BENEFITS

Philip Robson[1,2]

[1]GW Research Ltd, Porton Down Science Park, Salisbury SP4 0JQ, UK;
[2]Department of Psychiatry, University of Oxford, Warneford Hospital, Oxford OX3 7JX, UK

Potential mental health risks of cannabis: The endocannabinoid system is of particular interest in schizophrenia. CB_1 receptors are up-regulated, and CSF anandamide levels have been reported to be inversely proportional to symptom severity. Impaired CB_2 receptor functionality heightens vulnerability to psychosis. Epidemiological studies indicate a dose-related increased risk of psychosis in cannabis smokers which may be enhanced by early initiation of cannabis use. In healthy subjects THC can produce transient positive and negative symptoms of psychosis. However, many other recreational and prescribed drugs are also capable of causing transient psychosis, and no epidemiological link between increased national consumption of cannabis and subsequent schizophrenia prevalence has yet been demonstrated. One influential interpretation of the current data is that cannabis is a risk factor in an as-yet unidentified sub-group with an underlying genetic vulnerability to psychosis.

Short term effects on mood are common in cannabis smokers, but evidence linking its use to chronic depression or anxiety is unconvincing. The risk of psychological dependence is roughly equivalent to that of alcohol, and cannabis is the principal drug of abuse in almost a fifth of individuals entering US drug treatment programmes. Outcomes are generally poor.

Potential benefits of cannabinoid medicines in mental health disorders:

1. Schizophrenia: There is great unmet clinical need since current antipsychotics do not successfully target cognitive deficits or negative symptoms, and are only partially effective against positive symptoms in many patients. Evidence in humans is accumulating of the antipsychotic potential of cannabidiol (CBD), and interesting perspectives have been opened up by a series of studies employing functional magnetic resonance imaging (fMRI). The molecular basis for these effects remains speculative, and several hypotheses have been proposed. There is preliminary evidence that CBD and CB_1 receptor antagonists may benefit negative symptoms and cognitive deficit. Schizophrenia is also associated with chronic systemic inflammation, abnormal stress response, and serious metabolic abnormalities. All of these problems may be amenable to cannabinoid medicines.

2. Anxiety: Considerable evidence of an anxiolytic effect of CBD has accumulated, with significant improvements demonstrated in anxious patients and healthy subjects exposed to stressful tasks or stimuli. As with psychosis, interesting pointers to the brain mechanisms underlying these effects have been revealed through the use of fMRI.

3. Addiction: Human studies have demonstrated that cannabinoid medicines can be effective in reducing withdrawal symptoms and improving retention during detoxification from cannabis dependency. Laboratory studies have indicated that CBD reduces drug-seeking behavior in models of opioid and stimulant dependence.

4. Miscellaneous targets: Preliminary evidence suggests that cannabinoid medicines are worthy of further investigation in post-traumatic stress disorder, bipolar disorder, anorexia nervosa, and attention deficit hyperactivity disorder.

AMAZING MULTIPLE SCLEROSIS RECOVERY USING SATIVEX

Sarah Martin

27 Alspath Road, Meriden, Coventry

In March 2012 an MS patient began to treat her illness using Sativex. Until then she had been manufacturing her own cannabis oil but supply was small and varied. Treating her illness with standardised doses of Sativex, she has experienced great recovery from a relapse that destined her to live the rest of her life in a wheelchair. She walks using crutches and now and hopes for greater improvement using Sativex.

The talk will focus on the therapeutic effects of Sativex and will discuss the empirical experience of a patient in the short, medium and continuing long term phases of use.

In the initial two months after adopting Sativex as a daily medicine, it was found to cause some pain. For this reason, there was considerable doubt regarding its effectiveness at treating MS symptoms. However, knowing Sativex is a new medicine, it is important to record and share the recovery process.

After an initial introductory stage of two months, the patient found her doctor was able to give her a higher dose of Sativex each month. This resulted in a much quicker recovery where joint flexibility, muscle strength and balance were regained. The patient found she was able to walk using crutches and hopes to graduate to use walking sticks soon.

The medium phase has involved 30ml of Sativex in each weekly prescription. Exercise caused some pain but after each period of pain comes a period of greater muscle strength and joint flexibility. Spasms involving vision have also lessened allowing easier driving and reading.

Long term use of Sativex is ongoing and physical ability is constantly returning. Cannabinoids are enabling the body to function more healthily and repair itself. This extra health has meant greater mobility and socialising. Both these factors go a long way to repairing health as well. Increased movement enables exercise so that health may be maintained. This leads to some muscle pain that may suggest Sativex needs to provide further pain control. Would Sativex benefit from more THC or another cannabis compound? Extra pain relief needs to be found so that daily exercise is made easier.

THE POTENTIAL OF CANNABIS WHEN CONSUMED IN ITS NATURAL FORM

Tina Silva

Medical Campaign Director, NORML UK, *Medical Cannabis Patient UK*

Introduction: I have had problems with my health since birth. As a baby I contracted Whooping Cough and Scarlet Fever, Rheumatic fever around the age of 6. I have always had problems with my feet and legs, and wore corrective boots and callipers. During my teenage years my right knee started to spontaneously dislocate. At the age of 18, my knee would not relocate on its own, at the hospital an arthroscopy showed a rocky knee cap, prone to dislocation. After the birth of my daughter in 1994, all my previous symptoms became worse, and I also started to experience new symptoms. In 1996 I was diagnosed with Charcot-Marie-Tooth disease (CMT also known as hereditary motor and sensory neuropathy or peroneal muscular atrophy), In 1997, I had two major foot operations and a year later told I may never walk again. I have also been diagnosed with the following comorbid conditions: Primary parathyroidism, Postural orthostatic tachycardia syndrome (POTS, Ehlers–Danlos syndrome (EDS) (Also known as joint hypermobility syndrome type 3), Musculoskeletal Pain, Neuropathic Pain, Chronic Back Pain, Disk bulging, Osteopenia, Irritable Bowel Syndrome, High Cholesterol In 2000, after taking prescribed pharmaceuticals for years, I felt my body had received enough abuse, and I spent time in Portugal detoxing from pharmaceutical medication as I had become addicted to them. It was at this point I decided to use cannabis as medicine and started to grow my own to ensure I did not risk my health at any further from consuming poor quality or contaminated cannabis produced by criminal gangs. I currently cannot be prescribed Bedrocan or Sativex in the UK.

Methods Home grown Indica and Sativa cannabis, which was then prepared and consumed by myself, in the following ways: - Juicing raw cannabis, edible cannabis butter used in cooking, topical cream/balm and also inhalation using a vaporiser. My presentation will explain the positive health benefits I derive from consuming herbal cannabis in different ways, at different times.

Results: In 1997/98 I was told I would never walk again. Since consuming cannabis as medicine I am able to once again live a dignified and independent life. Cannabis eases my body, and minimises my pain enabling me to walk and to be as active and healthy as possible. It is for these reasons that using cannabis as medicine, despite its legal status, for me is an on-going conscious decision.

Conclusions: My decisions to not take prescribed medication, but instead to medicate myself with cannabis, is supported by the medical team who review my care annually, including my own GP, and professors specialising in Rheumatology, Neurology and CMT. They can all see, and have documented the tangible improvements in my mobility, pain management and general health.

STUDIES, SURVEYS AND STATISTICS: DO WE KNOW WHAT PATIENTS WANT?

Arno Hazekamp

Bedrocan BV; Leyden University, The Netherlands

Self-medication with cannabis is the most common way of using cannabinoids medicinally, despite the fact it is illegal in most countries. Consequently, there may be a lot to learn from the actual experiences of patients self-medicating with cannabis products worldwide. Outside the realm of modern medicine, patients find access to a wide range of cannabis varieties, administration forms and dosing regimens. Even during times and in places where scientists were not legally able to study the therapeutic effects of cannabis, large numbers of patients have kept experimenting with various forms of cannabis medicine. As a result, cannabis is a highly democratized medicine; on many aspects patients have more collective knowledge on the effects of cannabis and cannabinoids than scientists or physicians do. Patients communicate their experiences, recipes and recommendations through a large number of professional magazines, websites and even medical cannabis fairs. Medicinal cannabis has become a large and world-wide subculture that needs to be reckoned with.

In contrast, the absence of quality control or guidance by a trained physician may leave patients exposed to severe medical and legal risks. Evidence-based monitoring of the efficacy of cannabis on the indications for which it is used, and whether it is being used effectively and responsibly, is almost entirely lacking. While safety of cannabis is generally accepted to be within the range often deemed to be acceptable for other medications, clinical trials have not yet been able to supply a clear answer on what are supposed to be the 'real' medical indications for cannabis use. As a result, many current systems leave enough incentive for recreational users to act as pseudo-patients in order to obtain legal protection for using cannabis, creating considerable legal confusion. Finally, there is still much to learn about the risks of potential contaminations with pesticides, growth-enhancing chemicals, microbes or heavy metals. For all these reasons, physicians are often hesitant to play the role of prescriber or 'gate-keeper', and authorities find medicinal cannabis a confusion topic to discuss.

In order to find a balance between the benefits and risks of medicinal cannabis use, and build a bridge between patients' needs and the demands of modern medicine, a better exchange of information between patients and medical professionals is needed. Fortunately, there is a growing interest in performing scientific studies on patient populations, and to contribute to the understanding of cannabinoid-based medicine by asking self-medicating patients detailed questions about their experiences. This presentation will give an overview of the data that is available on the extent of medicinal cannabis use and the characteristics of patients involved in it.

THE EDUCATIONAL NEEDS OF PHYSICIANS REGARDING CANNABIS AND CANNABINOIDS

Mark A. Ware

Associate Professor, Anesthesia and Family Medicine, McGill University, Montreal, Quebec
Executive Director, Canadian Consortium for the Investigation of Cannabinoids

Advances in the basic scientific understanding of cannabinoid mechanisms of action, coupled with new approaches to clinical delivery of cannabis and cannabinoids and emerging clinical trial evidence of these compounds, are currently not part of standard educational curricula for health care professionals. Given the increasing availability of such approaches and emerging regulatory frameworks, there is a critical need to improve the transfer of this knowledge to the practitioners who have to make clinical decisions regarding this topic. This presentation will focus on efforts to identify the needs of such professionals, with a specific focus on physicians, and will outline some ongoing efforts to identify and address these knowledge gaps. Discussion will revolve around future collaborative mechanisms to raise the level of awareness of the risks and benefits of the medical use of cannabinoids with the goal of improving physician-patient interaction on this controversial topic.

The Educational Needs Of Physicians Regarding Cannabis And Cannabinoids

"THE CLINICAL MANAGEMENT OF THE PATIENT USING CANNABINOIDS"

Willy Notcutt

James Paget Hospital, Great Yarmouth, UK

Cannabinoids are new medicines to most physicians. Whilst they need education on the scientific background, they also need knowledge of practical aspects of use

- Preparing the Patient
  - Patient selection and assessment
  - Advice to patients
  - Dosing , titration and available medicine
  - Side effects
  - Dealing with the myths addiction, euphoria/high etc.
  - Caution with certain groups
  - The previous cannabis user
  - Driving
- Follow up
  - How soon?
  - Assessment of benefits and side effects
  - Side Effects
  - Psychiatric and Addiction matters
  - Long term management
  - Travelling to other countries
- Other Problems that occur
  - Patients who die
  - Costs of cannabinoids
- Physician issues
  - Results of clinical trials
  - Teaching other Doctors – Problems of opiate mis-prescribing
  - Problem of status of cannabis in community. Awareness of the local law.
  - Responsibility to advise users of illicit medicinal
  - Understanding the media and the politicians
  - Keeping to the Medicinal and Recreational apart
  - Problem of status of cannabis in community. Awareness of the local law.
  - Dealing with colleagues

WHERE ARE WE WITH THE USE OF CANNABINOIDS IN MULTIPLE SCLEROSIS?

Prof John Zajicek, PhD, FRCP

Chair Clinical Neuroscience,
Plymouth University Peninsula Schools of Medicine and Dentistry.

There is now convincing evidence that cannabinoids (either THC or combined THC and CBD) are effective in alleviating MS-related symptoms, especially muscle stiffness, spasms, pain and urinary disturbance. Getting to this point has been difficult due to substantial methodological problems in the interpretation of clinical trials because of issues of blinding, powerful placebo effects and problems in the measurement of patient-experienced symptoms. Even though we now have a licensed cannabinoid for treating MS-related spasticity, its use in some countries is restricted, as the limitations of health economic analysis do not allow for the complexity of patient experience in MS. This has led to rationing on the basis of perceived lack of cost-effectiveness.

We have also begun to explore the potential of cannabinoids to protect the nervous system, beyond symptom alleviation. This has generated a further set of methodological issues, more reflective of the state of trials in neurodegeneration rather than issues specific to cannabinoids. The contribution and understanding of symptom relief in disability reduction remains, as does the measurement of relevant clinical disability in MS and other conditions. Although the recent CUPID trial suggested no overall contribution of dronabinol to the slowing of progression in MS, there were major issues in the relative lack of deterioration in the study population, and intolerance to medication which make interpretation difficult. There was some suggestion of a treatment effect in less disabled people. Further studies need to take account of these findings, which again highlight the problems of trying to dissociate a treatment effect from the unwanted side effects of CB_1 receptor stimulation.

THE SATIVEX SOLUTION TO THE MEDICINAL CANNABIS CHALLENGE

Ethan Russo, MD[1, 2]

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[2]GW Pharmaceuticals, Porton Down Science Park, Salisbury SP4 0JQ UK

Introduction: Despite a long history of medical application, cannabis-based medicines were absent from the international pharmacopoeia for some seven decades until Sativex, an oromucosal extract of two cannabis chemovars expressing high titres of tetrahydrocannabinol and cannabidiol respectively, was approved in Canada in 2005 for treatment of central neuropathic pain in multiple sclerosis. Since that time, 19,500 patient-years of experience with this preparation has been gained in various diagnoses with regulatory approval in 22 countries, most often for treatment of spasticity in multiple sclerosis, but also for opioid-resistant cancer pain in Canada. This presentation will cover a wide range of topics related to the Sativex research programme and its results.

Methods: Current relevant literature was reviewed.

Results: International regulatory requirements for production of cannabis-based medicines are being harmonised, while those of the US Food and Drug Administration '*Botanical Guidance'* remain the most stringent. The Sativex research programme has addressed the particular challenges in producing a prescription pharmaceutical from cannabis that is at once, clinically relevant in its effects various diseases, while simultaneously demonstrates a very acceptable adverse event profile and demonstrably uniform biochemistry from batch to batch over many years. Various cannabis controversies will be examined, specifically: pharmacokinetics of cannabis and dose titration (wherein Sativex is absorbed in a time-frame that allows dose titration with low serum levels that avoid psychoactive side effects), whether cannabis randomised clinical trials are properly blinded (wherein Sativex has effectively performed in repeated analyses), the drug abuse liability of such medicines (wherein Sativex has a DAL equal to or lower than that of Marinol®/dronabinol), the regulatory scheduling of cannabis based medicines (Sativex is Schedule IV in the UK), their effects on cognitive function (Sativex has a benign profile), and distinctions between cannabis delivery techniques.

Conclusions: Sativex has demonstrated all the key components required of a modern pharmaceutical product that is uniform and consistent, efficacious and safe. Various controversies attendant to recreational cannabis usage have been successfully addressed by its composition and delivery technique.

THE PLACE OF HERBAL CANNABIS IN TODAYS' PHARAMCEUTICAL/MEDICAL PRACTISE

Tjalling Erkelens

Bedrocan BV, The Netherlands

Medicinal grade cannabis has been available on prescription in the Netherlands for ten years now. Bedrocan BV is currently the only grower contracted by the Dutch Ministry of Health to produce cannabis of standardized quality (as a pharmaceutical raw material) for this national program. The Office of Medicinal Cannabis (OMC) oversees the program and ensures a sufficient supply is available to patients, researchers, and for the development of cannabis-based medicine. Under its regulatory control the export of medicinal cannabis from the Netherlands has been possible since 2007, but only under certain restrictions with regards to duration and quantity.

The success of the Dutch program is reflected in the rising number of prescriptions and the increasing amount of cannabis prescribed to patients in the Netherlands. Since 2010 these numbers have doubled. The increasing scientific demand, the increase in export and the growing number of countries allowing medicinal cannabis to be prescribed to patients further underlines this success. The Czech republic and Norway have recently joined Italy, Finland and Germany in allowing the prescribing and import of medicinal cannabis. Several other European countries are investigating a possible change in regulations and laws that will allow patients access to medicinal cannabis.

Standardized herbal cannabis of pharmaceutical quality will , in its current raw form, not put an end to the (sometimes) hostile discussions that arose over the last few decades between patient(group)s, the medical community, regulatory bodies and politicians on this subject. These ongoing discussions have now unfortunately led to a great deal of distrust between these groups. For an important part this is due to a certain overlap between true medical users, and recreational users who see a chance to push for legalizing cannabis by medicalizing the substance.

The regulatory system for modern drugs has proven itself to be a good tool to control dangerous, addictive or highly attractive drugs. But over the years, increasing numbers of patient testimonies, together with several clinical trials with non-standardized cannabis have shown the effectiveness of cannabis in treating many conditions. In addition, clinical trials provide an undisputed way to test substances on their efficacy and effectiveness. Over the last ten years Bedrocan has worked on bridging the gaps between these groups by trying to pick the best of all these worlds.

On the basis of chemically standardized cannabis varieties, a recently developed and clinically tested placebo, and a newly developed dispenser (vaporizer) in combination with a dosing unit containing ground up herbal cannabis, Bedrocan and its partners have started the regulatory process of registering herbal cannabis for the treatment of chronic neuropathic pain. Clinical trials are expected to start in 2014.

Finally, also the subject of so-called "cannabis oil" and the mounting evidence on the efficacy of Cannabidiol (CBD) has lately become of utmost importance among patients and scientists. A study on the possibilities to produce a standardized and safe extract has been performed and a new cannabis variety containing approx. 9 % CBD and < 0.4% THC is ready to be made legally available in the Netherlands.

This presentation will give a comprehensive overview of achievements made so far and what is in the pipeline as far as Bedrocan BV is concerned.

CANNABIS OIL: -CHEMICAL EVALUATION OF AN UPCOMING CANNABIS-BASED MEDICINE-

Luigi L Romano[1], Arno Hazekamp[2]*

[1]Department of Pharmacy, University of Siena, Italy
[2]Plant Metabolomics group, Institute of Biology, Leiden University, The Netherlands
*: corresponding author

Introduction: Cannabis derived compounds are used by years for their palliative effects in cancer patients especially to inhibit chemotherapy-induced nausea and vomiting, stimulate appetite and inhibit pain [Guzmán, Nat. Rev. Cancer. 2003; 3(10):745-755]. In addition, preclinical evidence has shown cannabinoids to be capable, under some conditions, of inhibiting the development of cancer cells by various mechanisms of action, including apoptosis, inhibition of angiogenesis, and arresting the cell cycle [Calvaruso et al, Int. J. Oncol. 2012; 41(2):407-413; Valesco et al, Nat. Rev. Cancer 2012; 12(6):436-444.]. In recent years the captivating story of a former patient called Rick Simpson, who claims to have cured his skin cancer through repeated topical application of a concentrated cannabis extract also known as "Cannabis oil", has received increasing attention. According to his own recipe, hundreds of patients have been using the Cannabis oil for their selfmedication cancer-cure and describe the effects of the oil on websites dedicated to medicinal cannabis use, and through popular magazines, Youtube videos and other media. On this basis, the aim of this small study is to better understand the extraction methods and the composition of the new claimed anti-cancer treatment also known as Cannabis Oil.

Materials and methods: Cannabis plant material used in this study was of the variety "Bedrocan®" (19% THC w/w). Five different extraction protocols for the production of concentrates were assessed, these included: a naphtha and a petroleum-ether extraction according to instructions by Rick Simpson [Simpson 2008 (<http://www.youtube.com/watch?v=0psJhQHk_GI>), Simpson 2013 (<http://phoenixtears.ca/>)]; an ethanol extraction based on an authoritative Dutch website on Cannabis oil [Bruining 2013 (<www.mediwiet.nl>)]; and two olive oil extractions using different degrees of heating based on popular Youtube videos [Dr. Diane 2013 (<http://www.youtube.com/watch?v=vs66uyiH968>)].

All preparation methods consisted of a few simple steps: one or two extraction steps, separating plant material from solvent, and finally (in case of organic solvents) an evaporation step to produce a concentrate. For the ethanol extraction we also tested the effects of preheating (decarboxylation), treatment with activated charcoal and "winterization" (data not reported). After the extraction all the samples were diluted and analyzed through GC/FID, HPLC and ^{1}H-NMR analysis in order to detect the composition, cannabinoids and terpenes, and the residual solvent traces.

Results: When comparing five methods of Cannabis oil preparation, some interesting differences were observed between the resulting extracts. The most relevant differences were noted in the terpenes profiles Not so great differences were observed in the cannabinoids profile except for the two olive oil preparations that show a relevant increase in the cannabinoids peaks area.

Based on GC/FID and ^{1}H-NMR analysis, residual solvent traces still remain in the extracts especially for naphthalic extraction. We also performed a GC/FID analysis of a sample provided by a patient who makes the extraction following the Rick Simpson's method and the resulting data, in agreement with the ours, show a considerable amount of naphtha traces in the extract analyzed.

Conclusions: As extraction solvents for the production of Cannabis oil, ethanol and olive oil were shown to perform much better, extracting all terpenes and cannabinoids tested very efficiently and, additionally, these solvents are safe for consumption. Olive oil is cheap, not flammable or toxic and ethanol can be easily removed through evaporation. As a trade-off, however, olive oil extract cannot be concentrated by evaporation, which means patients will need to consume a larger volume of it in order to get the same therapeutic results. In a follow-up study on the use of Cannabis oils, there should be more focus on the characteristics and motivations of those who use it for self-medication.

THE PHARMACOLOGY AND THERAPEUTIC POTENTIAL OF PLANT CANNABINOIDS: GREAT EXPECTATIONS

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Recent research into the pharmacological actions of three non-psychoactive plant cannabinoids, cannabidiol (CBD), cannabidiolic acid (CBDA) and cannabigerol (CBG) has identified pharmacological actions of these phytocannabinoids that could well underlie a number of important potential therapeutic uses for each of them. More specifically, CBD has been found to display significant potency at enhancing the activation of 5-HT_{1A} receptors by a direct agonist, as indicated, for example, by its ability to produce such enhancement *in vitro* at 100 nM, a concentration well below any at which it has been shown to activate 5-HT_{1A} receptors directly (>10 μM). Its enhancement of 5-HT_{1A} receptor activation most likely explains its ability to prevent cerebral infarction, to reduce signs of anxiety and depression, to ameliorate cognitive and motor impairments and to decrease vomiting and signs of nausea in animals. It is possible too that CBD may be effective against negative symptoms and impaired cognition of schizophrenia because it enhances 5-HT_{1A} receptor activation. CBDA shares the ability of CBD both to induce such an enhancement and to decrease vomiting and signs of nausea in animals. Importantly, the dose-response curves of CBD and CBDA for the production of these effects are bell shaped, and CBDA is active over a much broader range of doses than CBD, and also displays much greater potency than this other phytocannabinoid. Further research is now needed to seek out the mechanism(s) by which CBD and CBDA produce their enhancement of 5-HT_{1A} receptor activation. Turning next to CBG, it possesses significant potency *in vitro* both at producing signs of α_2-adrenoceptor activation and, in contrast to CBD and CBDA, at blocking 5-HT_{1A} receptors. This has already prompted research leading to the further discoveries that CBG (i) produces apparent α_2-adrenoceptor-mediated analgesia in a mouse model of inflammatory pain and (ii) reduces phencyclidine-induced negative signs of schizophrenia in rats, suggesting that it may have therapeutic potential both as an analgesic and as an antipsychotic medicine. A fourth phytocannabinoid that has important potential therapeutic uses is Δ^9-tetrahydrocannabivarin (THCV). Thus, THCV is both a CB_2 receptor partial agonist and a CB_1 receptor antagonist, and so might ameliorate at least in part, symptoms of Parkinson's disease, systemic sclerosis, epilepsy, inflammation and inflammatory pain, obesity, liver damage, stroke, osteoporosis and nicotine and/or cocaine dependence/relapse, possibilities that are already supported for some of these disorders by results obtained with THCV in preclinical in vivo investigations. We have also recently obtained evidence that a metabolite of THCV, 11-OH-THCV, behaves as a reasonably potent CB_2 receptor neutral antagonist *in vitro* in the [^{35}S]GTPγS binding assay, just one possible therapeutic application for such an antagonist being the treatment of severe sepsis. It will be of interest to optimize this action of 11-OH-THCV by performing structure-activity experiments, since other compounds that have been reported to block CB_2 receptors, for example AM630 and SR144528, behave as CB_2 receptor inverse agonists rather than as neutral antagonists. Findings with CBD, CBDA, CBG THCV and 11-OH-THCV obtained by us and some of our collaborators will be presented.

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THE NEED FOR MECHANISMS: HOW MUCH DO THE PHYTOCANNABINOID AND ENDOCANNABINOID WORLDS OVERLAP?

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Endocannabinoids were discovered following the cloning of the two high affinity receptors for the major psychotropic cannabinoid component of *Cannabis sativa*, that is, THC. These binding sites are G-protein-coupled receptors (GPCRs) known as CB_1 and CB2. It was reasoned that such receptors would only have a "raison d'etre" if endogenous ligands for them, the endocannabinoids, in fact, did exist. However, we now know that endocannabinoids, which are lipophilic local mediators derived from arachidonic acid, the most studied of which are anandamide and 2-arachidonoyl-glycerol (2-AG), also interact, with high affinity and potency, with other receptors and ion channels. Furthermore, anandamide and 2-AG are accompanied in tissues by congeners which: 1) do not necessarily activate CB_1 or CB2, 2) have their own molecular targets among GPCRs, ion channels and nuclear receptors, 3) share with endocannabinoids some metabolic pathways andenzymes, and 4) are generally indicated as "endocannabinoid-like mediators" (ELMs). Finally, some oxidation e metabolites of anandamide and 2-AG have non-CB_1, non-CB2-mediated activities of their own. I have recently proposed that the ensemble of endocannabinoids, their oxidative metabolites, their receptors, ELMs, and the metabolic machinery necessary to regulate the levels of these mediators, be defined as the "endocannabinoidome".

Recent experiments have been focused at investigating the pharmacological actions and molecular targets also of other abundant cannabinoid components of *Cannabis*, such as cannabidiol (CBD), cannabichromene, cannabigerol, tetrahydrocannabivarin (THCV), cannabidivarin and their corresponding acids. At least some of these "phytocannabinoids" are in fact likely to contribute to the medicinal actions of *Cannabis* and could be more easily utilized in the clinic because devoid of psychotropic effects. Particularly, much is known about CBD and THCV mechanism of action. Whilst the former compound has low affinity for cannabinoid receptors, but interacts with a multitude of different receptors, THCV has been suggested to act as a potent neutral CB_1 antagonist, and a weak CB2 agonist. However, both compounds, unlike THC and like anandamide, activate the transient receptor potential vanilloid type-1 (TRPV1) channel and antagonize the transient receptor potential melastatin type-8 (TRPM8), channel, two important cation channels involved in pain perception but also other central and peripheral functions and pathological conditions. Furthermore, CBD, like some ELMs, was suggested to activate some peroxisome proliferator-activated nuclear receptors, and to antagonize the orphan GPCR, GPR55, which was proposed as an alternative receptor for endocannabinoids, some ELMs and synthetic CB_1 receptor inverse agonists. Finally, some members of a family of ELMs known as lipoaminoacids were shown to activate other orphan GPCRs, the activity of which may well be regulated also by plant cannabinoids.

In conclusion, the endocannabinoidome and the "phytocannabinoidome" might have much more in common than CB_1 and CB2 receptors, thus possibly lending some support to the hypothesis that mammals and the *Cannabis* plant may have coevolved, and providing further rationale for the investigation, in both preclinical and clinical settings, of non-THC cannabinoids.

THE MOLECULAR BASIS OF CANNABIS SENSITIVITY IN THE DEVELOPING BRAIN

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Introduction: Besides modulating neurotransmitter release and synaptic plasticity *via* a retrograde route of action in the adult nervous system, endocannabinoids have recently emerged as key modulators of neuronal development. Available data furnish the hypothesis that a continuum of endocannabinoid actions overarches the differentiation and postnatal modulation of particular synapses. My laboratory has contributed to the present understanding of the molecular and cell biology of endocannabinoid signalling in developmental contexts by describing the anatomical blueprint of endocannabinoid signalling networks, characterizing endocannabinoid-induced neurogenesis, cell migration, and axonal growth and guidance, and highlighting molecular hubs for endocannabinoid signal diversification and upstream control. However, it is unknown whether Δ^9-tetrahydrocannabinol (THC) can trigger a cannabinoid receptor-driven molecular cascade to disrupt neuronal specification, imparting permanent structural deficits to the developing cerebrum.

Methods: Neuroanatomy aided by quantitative analysis at the subcellular level, iTRAQ protein array profiling and biochemistry, mRNA quantification and neurophysiology in mouse models and *in vitro,* and in human fetal specimens were deployed to causally linking THC action at cannabinoid receptors to long-lasting cytoskeletal reorganization in cortical neurons, and to altered synaptic wiring of the cerebral cortex with modifications enduring into the adulthood of affected offspring.

Results: 1) We establish that repeated THC exposure erroneously times CB_1 cannabinoid receptor activation to rewire the fetal cortical circuitry. **2)** By interrogating the THC-sensitive neuronal proteome we identify Superior Cervical Ganglion 10 (SCG10)/stathmin-2, a microtubule-binding protein in axons, as a substrate of altered neuronal connectivity. **3)** We find SCG10 reduced in the hippocampus of midgestational (week 18-22) human fetuses exposed *in utero* to cannabis, defining SCG10 as the first cannabis-driven molecular effector of the developing cortical circuitry. **4)** CB_1 cannabinoid receptor activation recruits c-Jun N-terminal kinases to phosphorylate SCG10, promoting its rapid degradation *in situ* within motile axons and microtubule stabilization. **5)** In doing so, THC enables ectopic formation of filopodia and neurite branching.

Conclusions: Our data highlight key sites of neuronal vulnerability to phytocannabinoids in the developing cerebral cortex, and define the maintenance of cytoskeletal dynamics as a first-order molecular target for cannabis, whose imbalance can greatly limit the computational power of neuronal circuitries in affected offspring.

CB_2 RECEPTORS AS A PROMISING TARGET FOR DEVELOPING DISEASE-MODIFYING THERAPIES IN NEURODEGENERATIVE DISORDERS

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Cannabinoid CB_2 receptors have attracted a considerable interest as a promising target for developing novel neuroprotective and anti-inflammatory therapies in conditions of acute or chronic brain damage. This interest is based first on the lack of apparent side effects (e.g. psychoactivity) of those cannabinoids targeting selectively this cannabinoid receptor type. More importantly, its relevance as a neuroprotective target comes from the observation of up-regulatory responses of this receptor occurring in specific neural cells (mainly activated glial cells) in response to brain damage, responses that appear to be part of an endogenous neuroprotective response against excitotoxic, inflammatory, traumatic, oxidative and/or infectious stimuli. Such up-regulation of CB_2 receptors has been found in post-mortem tissues from patients, from experimental animal models, or from both, in acute (e.g. ischemia, brain trauma, spinal injury) or chronic (e.g. Alzheimer's disease and other related-dementias, Huntington's chorea, amyotrophic lateral sclerosis, multiple sclerosis, hereditary cerebellar ataxias and Parkinson's disease), and, more importantly, the pharmacological activation of these up-regulated receptors have been found to produce beneficial effects on neuronal survival in experimental models of most of these disorders. These effects were observed with cannabinoids that selectively activate the CB_2 receptor, but, in some cases, the effects could be enhanced using non-selective cannabinoids or combinations with other compounds targeting additional neuroprotective mechanisms (e.g. CB_1 receptors, PPAR receptors, nrf-2 signaling). The present lecture will review all the evidence accumulated so far that enables the CB_2 receptor to be considered as a promising target for neurodegenerative disorders, with emphasis in two of them: (i) autosomal dominant spinocerebellar ataxias, a disorder not investigated in relation with the cannabinoid system up to this year, and (ii) Parkinson's disease, for which the response of CB_2 receptors had remained elusive in the last years. In both cases, CB_2 receptors have been found to be up-regulated in glial elements in lesioned structures, but these receptors have been also identified in the specific neuronal subpopulations that degenerate in these disorders, i.e. Purkinje cells and nigrostriatal neurons, respectively, experiencing responses that may be used as specific markers of the progression of neuronal injury.

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"PHYTOCANNABINOIDS: A NEW OPPORTUNITY FOR PSYCHOSIS (PRECLINICAL EVIDENCE)"

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The search for safe and effective drugs to treat psychosis is hindered by the complex nature of this disorder, which is known to involve multiple brain neurotransmitters. The endogenous cannabinoids, a family of lipid messengers that target the same cell surface receptors engaged by D9-tetrahydrocannabinol (D9-THC) in marijuana, are considered to be among the most promising compounds for the treatment of psychosis.
Consistently, CB1 receptors are localized in brain areas relevant for the illness and several alterations of the endocannabinoid (EC) system have been reported either in humans as well as in experimental models of schizophrenia, although often with controversial results.
The well-known psychotropic effects of D9-THC, which are mediated by brain CB1 receptor activation, have greatly limited its clinical use. However, the plant Cannabis contains many cannabinoids with weak or no psychoactivity that, therapeutically, might be more promising than D9-THC. Among them Cannabidiol (CBD) has been one of the most studied compound and accumulating evidence either in rodents as well as in humans suggests that it can represent a new opportunity for the treatment of schizophrenia. This evidence prompted us to focus our attention on other phytocannabinoids, namely D9-tetrahydrocannabivarin (THCV), to assess its potential protective effect in an animal model of schizophrenia. We used a pharmacological model based on either acute or subchronic treatment with the NMDA receptor antagonist, phencyclidine (PCP), in order to reproduce specific endophenotypes of schizophrenia such as positive, negative and cognitive symptoms of the disease. Specifically, cognitive deficits were assessed in terms of recognition memory, negative symptoms in terms of social behavior and behavioral despair, whereas sensitization to PCP-induced hyperlocomotion and stereotypy was determined as a measure of positive symptoms. THCV at the dose of 2 mg/kg i.p. protected rats from the hyperlocomotion and stereotypy induced by acute PCP with the same potency as the atypical antipsychotic Clozapine. Furthermore THCV also counteracted cognitive impairment in the object recognition test as well as social withdrawal and behavioral despair induced by subchronic PCP treatment. Finally, to better characterize the mechanism of action of THCV, we tested whether its effect was mediated by the interaction with 5HT1A receptors by using a selective antagonist, WAY100635. We found that pre-treatment with WAY100635 at the dose of 1 mg/kg did not block the beneficial effect of THCV on PCP-induced hyperlocomotion but significantly reversed THCV-induced recovery of stereotypies, suggesting that 5HT1A receptors mediate the beneficial effect of THCV of stereotyped behaviors .. Ongoing experiments are aimed at investigating whether 5HT1A receptors are also involved in the effects if THCV on PCP-induced cognitive deficits and negative signs.
These preliminary data strongly favour further studies on other animal model of schizophrenia to verify the potentiality of THCV as antipsychotic drug.

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PHYTOCANNABINOIDS AS POTENTIAL ANTICANCER AGENTS

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Δ^9-Tetrahydrocannabinol (THC) and other phytocannabinoids inhibit cancer cell growth in vitro and in various animal models. This anticancer activity is dependent on the modulation of key signalling pathways that trigger cell death as well as other events such as inhibition of tumour angiogenesis, cell proliferation and cell invasion. During the last years, the work of our group in the field of Oncology has dealt mostly with glioma and breast cancer, focusing on (a) molecular mechanisms of action, (b) resistance mechanisms and (c) opportunities for combination-therapy approaches. These findings have provided preclinical proof-of-concept that cannabinoids could improve the clinical efficacy of classical cytotoxic drugs. Overall, such knowledge is, in our opinion, required for the optimization of preclinical cannabinoid-based therapies and the preliminary clinical testing that is currently underway. These issues, together with the most straightforward avenues to conduct clinical trials with cannabinoids in patients with glioblastoma multiforme and Her2-positive breast cancer, will be discussed in this presentation.

MAJOR CANNABIS CONSTITUENTS STIMULATE FRACTURE HEALING

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Bone fractures are amongst the most prevalent traumatic injuries, which usually involve prolonged immobilization and considerable discomfort. Most fractures heal by a process known as enchondral ossification. In this process initial bridging across the fracture gap is made by a cartilaginous callus, which mineralizes, resorbed by osteoclasts and replaced by a bony callus. The bony callus is further remodeled to form mature bone, which is similar to the pre-fracture tissue. Although cannabinoid ligands attenuate and rescue ovariectomy-induced bone loss, the effect of cannabis on the skeleton has not been reported so far. We tested the effect of Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) in a male rat model of mid-femoral fracture. THC and/or CBD were administered daily intraperitoneally for 2-8 weeks each at 5 mg/kg/day, commencing immediately after fracturing. Micro-computed tomographic analysis revealed that 2 and 4 weeks after fracture rats administered with THC or a THC and CBD mixture had reduced callus size resulting from a smaller unmineralized (cartilaginous) callus on week 2, leading to a smaller mineralized (bony) callus on week 4. These differences did not persist in the 6- and 8-week time points. The callus trabecular bone geometry was similar in the cannabinoid and vehicle administered rats at all time points. Biomechanical testing of the same femoral specimens showed a remarkable effect of CBD on the maximal load sustained by the specimen and particularly on the work to failure, which characterizes the callus toughness. This biomechanical effect was even more pronounced in animals treated with the THC and CBD mixture. The enhanced mechanical properties of the healing bone were associated with increased collagen crosslinking, while the material density (mineralization) of the extracellular matrix remained unchanged. These findings portray CBD as a potential enhancer of bone strength through a mechanism involving enzymes which catalyze collagen crosslinking.

MULTI-PROTECTIVE EFFECTS OF TETRAHYDROCANNABINOL (THC): ULTRA-LOW DOSES DEFEND THE BRAIN, THE HEART AND THE LIVER.

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We have previously shown that a single ultra-low dose of tetrahydrocannabinol (THC) protected the mice brain from a variety of insults including PTZ-induced seizures, CO-hypoxia, pentobarbital deep anesthesia and MDMA neurotoxicity. THC (0.002mg/kg, a dose that is 3-4 orders of magnitude lower than the doses that elicit the known acute effects of the drug), injected either 7 days before or 3 days after the insult, prevented the deteriorating effects of the insult as measured by a battery of cognitive assays 3-7 weeks following the insult. The same dose of THC induced long-lasting (7 weeks) biochemical effects in the brain, including the activation of ERK, CREB and BDNF in the hippocampus and frontal cortex.

A common feature of the various insults is the secondary activation of the innate immune system in the brain in response to the primary neuronal damage. We tested, therefore, whether THC is able to protect the brain from LPS-activation of the innate immune response. Intraperitoneal injection of the bacterial lipopolysaccharide (LPS) induced cognitive deficits that lasted for at least 7 weeks. A single injection of THC 0.002mg/kg either 48 hrs before, or 7 days after LPS prevented this long-term cognitive damage. The protective effect of THC was blocked by SR141716A, but not by SR144528, indicating the involvement of CB1 receptors. THC also attenuated the long-lasting (7 weeks) LPS-induction of cyclooxygenase-2 (COX-2) in the hippocampus and frontal cortex.

The innate immune system participates in, and contributes to, the development of damage in other organs as well. We therefore tested whether the same ultra-low dose of THC protected the mice heart from ischemic damage. Myocardial infarction (MI) was produced by coronary artery ligation and its morphological, functional and biochemical outcomes were studied 24 hrs later. MI produced a necrotic area (detected by TTC staining) that was infiltrated by neutrophils (observed by immunohistology), deteriorated functional shortening of the heart (as measured by echocardiography) and elevated the level of troponin T in the serum. All these parameters were significantly improved by THC (0.002mg/kg) injected either 2 or 48 hours before MI.

The ultra-low dose of THC was similarly effective in protecting the liver from ischemia-reperfusion (I/R) damage. A single injection of THC attenuated the necrotic area, suppressed caspase-3 activation, eliminated the expression of IL1a and TNFalpha in the hepatic tissue and reduced the level of liver enzymes in the serum.

The relevance of these findings to clinical conditions such as stroke, heart attack and liver transplantation will be discussed.

CANNABINOIDS IN PAIN AND PALLIATIVE CARE

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The endocannabinoid system and the cannabinoid receptors are involved in the central modulation of pain. The CB1 receptor, like the opioid receptor, is found in areas of the brain that modulate nocioceptive processing. Cannabinoid analgesic effects are not blocked by opioid antagonists suggesting distinct pathways. Animal models have suggested synergy between opioids and cannabinoids in relief of pain. CB1 and CB2 agonists also have peripheral analgesic actions. In addition, cannabinoids and other components of *Cannabis* species likely impact pain through anti-inflammatory effects.

Peripheral neuropathy is a painful condition that is not responsive to opioid analgesics. Animal models have suggested a role for cannabinoids. To date, numerous clinical trials have been conducted with inhaled cannabis products investigating the effectiveness in a number of neuropathic conditions. Most of these trials were funded by the University of California Center for Medicinal Cannabis Research. Remarkably, the studies all found a benefit to cannabis over placebo in neuropathic pain conditions with a number needed to treat consistently centering around 3.5 in all of the studies. A small pilot study of nabiximols (Sativex), the whole-cannabis extract medicine with a standardized THC:CBD ratio, in peripheral neuropathy also demonstrated effectiveness. Clearly cannabinoids appear effective in this difficult to treat clinical condition. Studies of nabiximols in pain associated with multiple sclerosis led to its approval in Canada and a number of European nations and nabiximols has also shown promise in patients with cancer-related pain.

To further investigate the suggestion from animal studies that cannabinoids and opioids may be synergistic, we initially conducted a classical pharmacokinetic interaction study to verify safety and evaluate the effect of adding vaporized cannabis to a stable dose of long-acting opioids. Although no adverse of clinically significant pharmacokinetic interactions were seen, there was a suggestion of increased relief of pain with the combination. Our group is currently funded to investigate a vaporized THC:CBD *Cannabis* strain in patients with sickle cell disease with chronic pain on a stable opioid dose; however the US Food and Drug Administration is requesting additional safety data on inhalation of cannabidiol before allowing the trial to proceed.

In addition to pain, patients with terminal illness, especially cancer, often face other debilitating symptoms that may be responsive to cannabinoid medicines. These include nausea and vomiting, anorexia, insomnia, anxiety and depression. Trial results will be reviewed.

CANNABINOID AND TERPENOID PROFILING OF MEDICINAL CANNABIS IN CALIFORNIA

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Introduction: Hundreds of *Cannabis Sativa L.* strains are presently available on the medical cannabis market in California. Over the years, numerous cultivars have been introduced into the state and many more new varieties, often called strains, have been developed by breeders and brought onto market under colorful varietal names such as "OG Kush," "Skunk," "Lemon Haze," "Jack Herer," etc. In California medical cannabis is recommended by physicians for a wide variety of conditions. However, patients typically receive no direct recommendation as to which variety may be most effective for their particular condition. In order to answer this question, it is first necessary to chemically classify cannabis varieties based on active constituents or chemotypes. Then, it may be possible to link certain cannabis chemotypes with specific conditions. The aim of this study was to examine a number of samples from selected varieties, analyze their cannabinoid and terpene profiles, and determine whether different varieties have consistent, distinct chemotypes.

Methods: Internally validated HPLC-UV and GC-FID methods were used to quantify both cannabinoids and terpenes respectively. THCA, CBDA and CBGA along with over 35 different terpenes were analyzed from the different cannabis flower samples. Four different named varietals were purposefully acquired across both the northern and southern parts of California and compared for chemotype similarity. Principal component analysis (PCA) was used to analyze the data and provide visualization of the results.

Results: Terpene profiles analyzed by PCA resulted in identifying four broad chemotypes corresponding to the four named varieties. However, a number of samples, named the same variety differed chemically from other samples with the same variety name indicating a lack of consistent quality control in the marketplace. The most common terpenes detected were β-caryophyllene, limonene, terpinolene and myrcene. Specific characteristics could be discerned in some varieties, such as Jack Herer, which was unusually high in terpinolene, and Sour Diesel, which was notably low in β-caryophyllene and high in limonene.

Conclusions: A new system of cannabis identification based on chemotaxonomical methods will need to be established in order to accurately verify the particular identity, or name, of a unique variety. Cannabinoid and terpenoid profiling with principal component analysis has been shown to be useful in elucidating distinctions between varieties in the California medicinal cannabis market. However due to lack of quality control more efforts are needed to better identify and name varieties properly in order to clear up confusion among patients and health care providers.

OPEN FORUM AND IACM MEMBER INPUT: WHERE ARE WE GOING AND HOW DO WE GET THERE?

Franjo Grotenhermen

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In this forum we would like to discuss future steps of the IACM with regard to the advancement of the aims of the association and of the advancement of the IACM as an association. Any input, suggestions, ideas, observations and critics are welcome.

The aims of the IACM are described in the statutes as follows: *"The aim of the association is to advance knowledge on cannabis, cannabinoids, the endocannabinoid system, and related topics especially with regard to their therapeutic potential. The mission is achieved in particular through the following actions:*

1. Support for research into cannabis products and the endocannabinoid system;
2. Promotion of exchange of information ...;
3. Preparation and dissemination of reliable information ...;
4. Monitoring and documentation of national and international developments ...;
5. Co-operation with other organisations and associations ..."

The IACM was founded in 2001 and after 12 years we see considerable advancements in the aims of the association. IACM members have been considerably involved in this process and the IACM itself may have played a small role in it - together with many others, individuals, societies, organisations and pharmaceutical companies - by organising scientific meetings, by bringing patients and scientists together, by co-operations with other associations in the field, by publishing the journals "Journal of Cannabis Therapeutics" (edited by Ethan Russo) and "Cannabinoids" (edited by Franjo Grotenhermen), by providing further information on our website and by informing the public by the IACM Bulletin in six languages on scientific and political developments in the field. The IACM used its international outreach for example for an international survey on the medical uses of cannabis in different languages or for the support of the Medical Cannabis Declaration.

However, we must also acknowledge that today **most people in the vast majority of countries in the world still have no legal access to the medicinal benefits of cannabinoids and cannabis**, and punishments for illegal medicinal use of cannabis in many countries without any or very limited legal sources are still harsh, even in many European countries. In some large countries such as Russia and China, and in many other countries of Asia, Africa and Latin America the knowledge among health professionals and possibilities for patients are still very limited.

The IACM was always a small association consisting of scientists with a high interest in the therapeutic uses of cannabis and cannabinoids, and of patients and other people with a global perspective with regard to political developments in this area. It is our aim in the near future to increase the benefits from a membership in the IACM and to easier open the door for newcomers. Two ideas, we want to realise in the near future is making the records of the talks of the 2013 IACM Conference on Cannabinoids available to members on our website and creating internal IACM mailing lists for members, one for scientists and other regular members in English, two for patients and other associate members in English and Spanish. This allows high-quality exchanges between members and **strengthens the feeling of being a member of this highly respected and unique association of wonderful people.**

THE ROAD AHEAD FOR CANNABIS RESEARCH

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I have worked on cannabinoids for 50 years and I am glad to note that research in this field – like in all active fields - incessantly changes directions. It started – quite late compared to other illicit drugs – by the badly needed elucidation of the chemical nature of the Cannabis plant. This phase was mostly completed during the 1960's and early 1970's. This advance attracted quite a few groups working on pharmacology and biochemistry. Then we learned much about the activity and metabolism of THC and cannabidiol and initial clinical trials were initiated. With the discovery of the cannabinoid receptors, the endogenous ligands and the pathways of their syntheses and metabolism in the 1980's and 1990's, the endocannabinoid field became a well established niche of classical biochemistry/physiology/pharmacology with a large number of groups looking at various aspects. Over the last 15 years we have learned much about endocannabinoid involvement in a myriad of body processes.
Where now? I assume that in the next decade we shall see novel findings in some of the following directions:

1. **CB2 receptor-mediated effects.** It seems possible that the CB2 receptor is part of a general protective system. We have speculated that "The mammalian body has a highly developed immune system which guards against continuous invading protein attacks and aims at preventing, attenuating or repairing the inflicted damage. It is conceivable that through evolution analogous biological protective systems have evolved against nonprotein attacks? There is emerging evidence that lipid endocannabinoid signaling through CB2 receptors may represent an example/part of such a protective system" As CB2 activation does not cause the psychoactive effects produced by CB1 agonists, I expect that we shall novel research on CB2 in essentially all physiological systems.

2. Fatty acid-amino acid entities (FAAA's) and related endogenous materials. The presence of huge numbers of endogenous compounds of these types cannot be a 'mistake of Nature'. Although these compounds (at least those investigated) do not bind directly to the cannabinoid receptors, the activities of some of them seem to be somehow associated with the endocannabinoid system. But most of their physiological roles are yet unknown. Their investigation may lead to important findings in many areas. Are they also associated with molecular psychology?

3. Cannabidiol (CBD). A long list of therapeutically positive effects are attributed to the non-psychoactive CBD, including anti-anxiety, anti-epileptic and anti-schizophrenic effects in patients. Although many of the mechanisms of CBD action have been elucidated, its wide range action is still a riddle. Are we missing something? Are there endogenous molecules with CBD-like effects?

The above and other unsolved problems in the cannabinoid field will be discussed.

Posters

CANNABIS IN THE THIRD AGE –EXPERIMENTAL THERAPY AT A NURSING HOME

Moshe Geitzen[1], Zach Klein[2], Lihi Bar-Lev Schleider[3], RNA Inbal Sikorin[4]

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"Hadarim" Nursing Home was established in 1994 at Kibbutz Na'an (Israel) and comprises of 36 beds lisenced by the Israeli Health Ministry. Some of the residents are lucid/alert while others are not and require comprehensive assistance with Activities of Daily Living (ADL). The therapuetic process using Medical Cannabis (23% THC) was initiated a year ago, in 2010.

During a period of 4 years, the selected species, Medical Cannabis (23/30% THC), was frequently tested at the laboratory of Professor Refael Meshulam by Dr. Lumir Hanus, at The Hebrew University of Jerusalem.

The species contains 23% THC.

We use clean minced flowers to be used by Vaporizer or the like, which produces smoke. Additionally, we use filtered medical cannabis powder, containing 30% THC.

In the Cannabis, there are over 460 active substances and up to 100 cannabinoids with therapuetic affect, among them the major ones are **THC**: psycoactive substance, with palliative effect over pain, nauseas, and vomiting. **CBD**: the major non-psychotropic substance, with anti-epileptic, anti-inflammatory, anti-emetic, muscles release, anxiolytic, anti-psychotic, and neuro-protective effects. Today, the cannabis in Israel is recognised as a treatment for HIV and ALS patients, Crohn's disease, malignancy at the chemical and therapy stages, any terminally ill patient, chronic pain, Multiple Sclerosis (MS) with spastic component, glaucoma, and Tourette patients.

In the history, for years cannabis was vaporized to treat more complex conditions. Due to the growing population of elders in the world, age-related multiple problems and medications, and severe suffering of a large portion of patients in their last years of living, we must utilize the main benefits of cannabis: lessened adverse reactions, mainly the fatal ones, simultaneouse effect over several problems, several means of administration – smoking, vaporization device, powder, oil, and local ointment.

The goals of our work are to monitor patients treated with cannabis and evaluate its influence over pain, appetite, sleep, mood, ADL, and quality of life.

Methods: 19 nursing patients were treated with cannabis during a year with supervision over pain with a pain scale, weight and blood-protein levels, sleeping chart, Cornell Scale for Depression in Dementia (CSDD), and Ashraf's test for Spasticity. The cannabis was administered by self-smoke, vaporization device, and powder, with individual dosages ranging from the start-point of 0.08 gr. in powder and 0.25 gr. in smoking/vaporization and a gradual increase of up to 3 gr. per day.

Results:

Polypharmacy Treatment – of the 18 subjects, among 13 patients (72.2%) medications were reduced with an average of 1.7 medications per day.

Nutritional measurements:

Weight – some of the patients lost weight while others gained weight. All subjects achieved proper weight and maintain it.

Albumin – all of the patients maintained normal albumin level.

Total Protein – all of the patients maintained normal protein level.

Spasticity Assessment – according to Modified Ashworth Scale, a 50% improvement in spasticity.

THE ANTITUMOR POTENTIAL OF THE ENDOCANNABINOID REUPTAKE INHIBITOR OMDM-2 AND ITS COMBINATION WITH CURCUMIN

Ramy Ammar[1*], Mona El-Azab[2], Yasser Moustafa[2] and Gudrun Ulrich-Merzenich[1]

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Introduction: Direct cannabinoid agonists can reduce tumor growth and progression in preclinical studies and animal models. However, their clinical use is limited due to their psychotropic side effects mediated by the central cannabinoid type-1 receptor (CB-1). An inhibition of the endogenous cannabinoid reuptake at the site of tumor growth might prolong the selective antitumor activity and avoid psychotropic side effects.

Methods: Four different sets of experiments were carried out: (**I**) Ehrlich ascites carcinoma bearing mice (n= 70) treated either with (1) OMDM-2 (5 mg/kg, i.p.), (2) R-Methanandamide (R-Met) (0.5 mg/kg, i.p.), a direct cannaboid agonist, (3) NIDA 41020 (0.7 mg/kg, i.p.), CB-1 receptor blocker, (4) R-Met + NIDA 41020, (5) OMDM-2 + NIDA 41020, or (6) Carboplatin (5 mg/kg, i.p.) were evaluated for tumor volume, mean survival time and increase in the life span (%). (**II**) Time course measurements of tumor weights were observed on days 7, 14, and 21 post-inoculation (n= 147, 21/ group). Hematological parameters were investigated on day 14. (**III**) The antiproliferative activity of OMDM-2 against MCF-7 human breast cancer cells was investigated *in vitro* by a Resazurin based assay. (**IV**) *In vitro*, the antagonistic, additive or synergistic effects of OMDM-2 and curcumin were determined by the Isobolmethod.

Results: (**I**) In mice, both OMDM-2 and R-Methanandamide significantly impeded tumor weights and volumes ($p<0.05$) at all time points. Furthermore, OMDM-2 significantly increased the mean survival time (37±4.3 vs 59±4.9 days) and also increased the life span of treated animals by 59.5% compared to the control group. After 14 days of inoculation, OMDM-2 was able to reverse changes observed in the hematological parameters after tumor inoculation. The combination of OMDM-2 or R-Meth with NIDA 41020 counteracted all previous effects in the R-Met-treated group but not in the OMDM-2 group. (**II**) *In vitro*, OMDM-2 reduced the viability of MCF-7 dose dependently with an IC_{50} of 9.3µM. NIDA 41020 (0.2µM) reversed the growth inhibitory activity of lower concentrations of OMDM-2 (2.5µM, 5 µM), an effect which was not observed with higher concentrations (20µM to 80µM). (**III**) Curcumin alone reduced the viability of MCF-7 cells dose dependently with an IC_{50} of 26.7µM. A 1:1 combination of OMDM-2 and Curcumin reduced the IC_{50} to 0.3 µM.

Conclusions: OMDM-2 showed *in vivo* (mice) an antitumor activity and *in vitro* (MCF-7) an antiproliferative activity. *In vivo*, the effect was not mediated by the CB-1 receptor. The combination of OMDM-2 and curcumin synergistically reduce cell viability of MCF-7 cells. Strategies combining molecules targeting the endocannabinoid system and natural anticancer agents should be further investigated as an addition to the next generation treatment for cancer.

A QUANTITATIVE DETERMINATION OF CANNABINOIDS IN PLASMA AND URINE USING GAS CHROMATHOGRAPHY-MASS SPECTROMETRY.

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Cannabis sativa L. is one of the most widely used illegal drug but it there are great expectations around the possible use in medicine due to the high therapeutic potential of cannabis. As a result, a big deal has been created between strong advocates who think that cannabis is a harmful drug by itself or as derivative and therefore, must be prohibited, and those who believe in the beneficial pharmacological effect. In this work we have tried to develop a reliable method for the determination of cannabinoids in plasma and urine. The aim of the study is to develop a chromatographic method in order to monitor some cannabinoids present in biological fluids from patients with chronic diseases which consume cannabis.

Methods: A quantitative method for the determination of cannabinoids (CBD, THC, CBN, THC-OH,THC-COOH) in plasma and urine has been developed. Solid Phase Extraction (SPE) has been elected as the extraction method for the compounds of interest employing, Strata C18 (200mg). The derivatization was performed using 1:1 EtAc : N,O-bis(trimethilsilyltrifluoroacetamide (BSTFA, 1% trimethylclorosilane) at 70 ºC for 30 minutes. For the determination of the cannabinoids a GC-MS method was developed.
In addition to this method, a SPME approach is being optimized based on the fiber absorption and on-fiber derivatization before being injected in the GC-MS setup.

Results: Employing the previously mentioned SPE method the analytes of interest have been recovered with minimal interference and the obtained recoveries were high. Furthermore, it has been confirmed that there is no matrix effect. The extraction procedure is repetitive and reproducible. Besides, the derivatization conditions are very appropiate for the completely derivatization of cannabinoids.

Conclusions: A reliable method for the determination of cannabinoids in plasma and urine has been carried out and can be a very useful tool for routine analysis in patients who consume cannabis.

Acknowledgements: The authors acknowledge Luis Bartolomé from SGIKER (UPV-EHU) for his collaboration in the SPME analysis and Ganjazz Art Club (Donostia) for providing marihuana samples.

CANNABINOID FINGERPRINTING OF 31 *CANNABIS SATIVA L.* VARIETIES USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Despite being illegal in many countries, the use of *Cannabis sativa L.* as a medicinal plant is widespread among the world. However, this use of cannabis is still far away from being medically controlled. Therefore, it is essential to study the distribution of the cannabinoids in different varieties in order to, first, establish the varieties profile and the similarities/differences between them and, second, to be able to dispense the correct varieties for the symptom alleviation and study potential interactions among the cannabinoids.

Methods: In this work, we have used a simple, rapid, highly sensitive and specific method for the analysis of cannabinoids from 31 cannabis varieties. They were extracted by Supercritical Fluid Extraction (SFE), at previously optimized conditions. Extracts were directly analyzed by means of High-Performance Liquid Chromatography tandem Mass Spectrometry (*HPLC-MS*/*MS*) and atmospheric pressure chemical ionization (APCI). Separations were obtained using a C18 column (length 2.1x100 mm, i.d. 2.6 μm). For quantification, the two most abundant MS-MS transitions of the analyte and the internal standard were monitored. For qualitative determination, the two most important peaks of the samples (THC-A and THC) were sent to waste before entering to the analyzer; thereby the samples were injected less diluted in order to find more cannabinoids. Statistical data treatment was used to assess the differences between varieties of *Cannabis sativa L.* by principal component analysis (PCA).

Results: 6 cannabinoids were quantified (THCA, THC, CBD, CBN, CBG and THCV) and more than 30 were identified. The cannabis varieties were chemically discriminated by PCA.

Conclusions: These results can help studies that want to analyze potential interactions of the cannabinoids or the therapeutic effectiveness of these varieties.

Acknowledgements: The authors are grateful to GANJAZZ ART CLUB for supplying the plant material used in this study and to IDOKI SCF S.L. for their support. O. Aizpurua and J. Omar are grateful to the Basque Government for their PhD fellowship.

Δ^9-TETRAHYDROCANNABINOL-11-OIC ACID, A UBIQUITOUS YET UNDERRESEARCHED CANNABINOID. AN OVERVIEW OF THE LITERATURE

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Synthetic Δ^9-tetrahydrocannabinol (THC), pharmaceutical grade herbal cannabis as well as formulations of well-defined cannabis extracts are available as registered medicines in several countries. The pharmacodynamics and pharmacokinetics of THC in animals and man have been extensively studied. It is now understood that the main central and peripheral pharmacological effects of THC are mediated by CB1 and CB2 cannabinoid receptors. The pharmacology of 11-hydroxy-THC, the primary oxidative metabolite of THC, is similar to the parent cannabinoid. Further cytochrome P450-mediated metabolic oxidations of the psychoactive 11-hydroxy-THC lead to the less lipophilic and non-psychoactive 11-nor-9-carboxy-THC (THC-11-oic acid, THC-COOH).

The pharmacokinetics of THC-COOH, discovered in the early 1970s, has been studied thoroughly. This abundant acid metabolite is now an established urinary marker of cannabis consumption in forensic, clinical and environmental analyses. Depending on the route and frequency of administration of THC-containing preparations, THC-COOH is present in blood plasma typically in the 3–100 ng/mL concentration range (0.01–0.3 nM). In urban sewage, the excreted acid metabolite has been detected at the tens of ng/L level. Surprisingly, however, data on the biological activity of this ubiquitous metabolite are scarce. (A homologous isomer of THC-COOH, ajulemic acid, was developed as an analgesic and anti-inflammatory cannabinoid (reviewed by *Burstein, AAPS J. 2005; 7: E143-E148*)). A few published studies have examined the effect of THC-COOH on the biosynthesis of prostaglandins and other eicosanoids, on capsaicin-sensitive sensory nerves, on the multidrug transporter P-glycoprotein, on the cannabinoid and estrogen receptors *in vitro*, as well as its anti-cataleptic, analgesic, platelet-activating factor inhibitory and anti-inflammatory activities *in vivo*; THC-COOH has also been reported to block certain behavioural effects of THC in rodents. Several patents also claim various therapeutic uses of cannabinoid carboxylic acids.

This presentation will review the literature on the reported pharmacological effects of THC-COOH. It also advocates further studies to reveal any potential involvement of this abundant phytocannabinoid metabolite in the complex pharmacology and in the proven therapeutic effects of THC-containing preparations.

EFFECTS OF Δ^9-TETRAHYDROCANNABIVARIN AT SEROTONINERGIC 5-HT_{1A} RECEPTORS, AND OF ITS 11-HYDROXY-METABOLITE AT CANNABINOID CB_1 AND CB_2 RECEPTORS

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Recently, we obtained evidence that cannabidiol (CBD) and its immediate precursor cannabidiolic acid (CBDA) display significant potency at enhancing the ability of the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT, to stimulate [^{35}S]GTPγS binding to rat brainstem membranes, and that both CBD and CBDA suppress vomiting in shrews and nausea-like behavior in rats by somehow augmenting the activation of 5-HT_{1A} receptors in the brainstem by endogenously released 5-HT (1, 2). We have also discovered that another constituent of *Cannabis*, cannabigerol (CBG), can interact, both *in vitro* and *in vivo*, with 5-HT_{1A} receptors, in this case opposing their activation with significant potency (3, 4). Here, we report evidence that Δ^9-tetrahydrocannabivarin (THCV), shares the ability of CBD, CBDA and CBG to interact with 5-HT_{1A} *in vitro*. The *in vitro* pharmacology of a metabolite of THCV, 11-OH-Δ^9-THCV, at both cannabinoid CB_1 and CB_2 receptors, is also described.

Methods: We performed: (a) [^{3}H]CP55940 displacement binding assays with MF1 mouse whole brain or hCB_2 CHO cell membranes, and [^{3}H]8-OH-DPAT displacement binding assays with MF1 mouse whole brain membranes; (b) [^{35}S]GTPγS binding assays with MF1 mouse whole brain, Sprague-Dawley rat brainstem, hCB_2 CHO cell or MF1 mouse spleen membranes, using methods we have described previously (3, 5). Mean apparent K_B values for 11-OH-Δ^9-THCV (1 μM) were calculated by Schild analysis.

Results: We found that in MF1 mouse whole brain membranes, both Δ^9- and Δ^8-THCV (100 nM) significantly increased the ability of the well-established 5-HT_{1A} receptor agonist, 8-OH-DPAT, to stimulate [^{35}S]GTPγS binding to mouse whole brain and to rat brainstem membranes. Furthermore, when tested in displacement binding assays using mouse whole brain membranes, Δ^9-THCV potently, but only partially, displaced [^{3}H]8-OH-DPAT from specific binding sites (5-HT_{1A} receptors). Moreover, when tested alone, 11-OH-Δ^9-THCV, (1 nM to 10 μM) did not affect [^{35}S]GTPγS binding to either mouse brain or hCB_2 CHO cell membranes. Also, at 1 μM, 11-OH-Δ^9-THCV induced a rightward, but not a downward, shift of the log concentration-response curve of CP55940 in mouse brain, hCB_2 CHO cell and mouse spleen membranes, thus behaving as an apparent CB_1 and CB_2 "neutral" antagonist.

Conclusions: (a) THCV can increase the ability of the 5-HT_{1A} receptor agonist, 8-OH-DPAT to stimulate [^{35}S]GTPγS binding to mouse whole brain and rat brainstem membranes, and potently, but only partially, displaces [^{3}H]8-OH-DPAT from specific binding sites in MF1 whole brain membranes; (b) 11-OH-Δ^9-THCV may be an important lead compound for a much needed *neutral* CB_2 receptor antagonist.

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THE NATURAL PRODUCT MAGNOLOL AS A LEAD STRUCTURE FOR THE DEVELOPMENT OF POTENT CANNABINOID RECEPTOR AGONISTS

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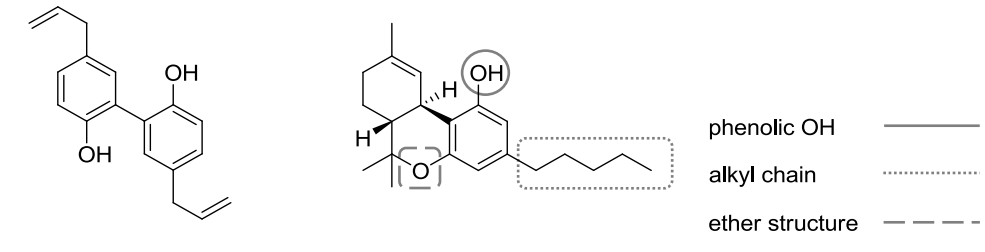
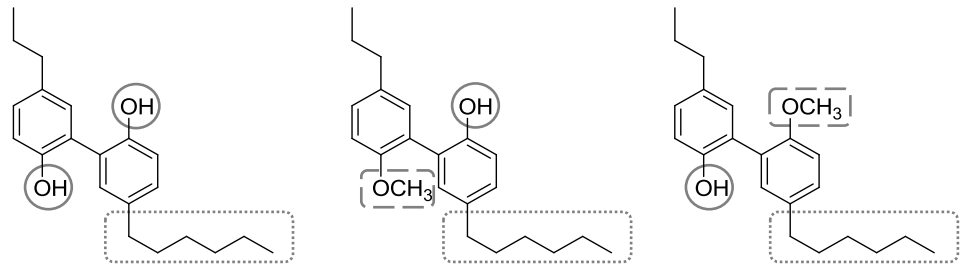
Recently we discovered that a bark extract of *Magnolia officinalis*, which has been used in traditional Chinese medicine (TCM) for the treatment of insomnia, anxiety disorders and allergic diseases, exhibits cannabinoid (CB) receptor agonistic effects. The main active constituents of *Magnolia officinalis* bark were shown to be the biphenylic neolignans magnolol, honokiol, and to a minor extent 4'-O-methylhonokiol. We could show that these biphenylic compounds interact with CB receptors. The main metabolite of magnolol, tetrahydromagnolol, was shown to be of even higher potency (20-fold) at the CB_2 receptor compared to its precursor (Rempel et al., *ACS Med Chem Lett* **2013**, *4*, 41-45). The simple and drug-like structure of the compounds which possess a low molecular weight and no chiral centers prompted us to develop analogs with improved agonistic properties. The new compounds were investigated in radioligand binding studies and functionally characterized at human CB_1 and CB_2 receptors. All compounds were additionally tested for activity at the related orphan receptors GPR18 and GPR55 to investigate their selectivity. Compared to the natural scaffold magnolol a 330-fold increase in CB_1 and a 60-fold increase in CB_2 receptor affinity could be achieved (Fuchs et al., *PLOS One* **2013**, in press). The new compounds were shown to be, in general, highly selective for CB receptors, in contrast to many available CB ligands, which were shown to interact with GPR18 and/or GPR55 as well. However, we could demonstrate that minor modifications can increase the compounds' potency at GPR55. Further optimization of this class of compounds towards GPR55 antagonistic activity may lead to the development of potent and selective GPR55 antagonists.



magnolol
K_i (µM) CB_1: 3.15 CB_2: 1.44
EC_{50} (µM) CB_1: 18.3 CB_2: 3.28

THC



K_i (µM) CB_1: 0.145 CB_2: 0.0294
EC_{50} (µM) CB_1: 0.829 CB_2: 0.159

K_i (µM) CB_1: 0.00957 CB_2: 0.0238
EC_{50} (µM) CB_1: 0.159 CB_2: 0.0385

K_i (µM) CB_1: 0.313 CB_2: 0.281
K_B (µM) CB_1: 0.159 EC_{50} (µM) CB_2: 0.595
IC_{50} GPR55 (µM): 3.25

RADIOLABELLING AND BIODISTRIBUTION STUDIES OF POTENTIAL RADIOLIGANDS FOR PET-IMAGING OF CANNABINOID RECEPTOR TYPE 2 (CB2)

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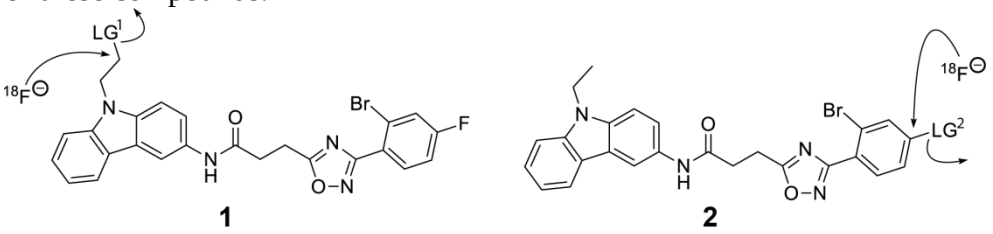
The level of expression of cannabinoid receptors type 2 (CB2) in healthy and diseased brain has not been fully elucidated. Furthermore, there is interest in the regional expression of CB2 in brain. Positron emission tomography (PET) is a technique, which allows monitoring of very low amounts of radiolabelled compounds in living organisms and, thus, is been used as diagnostic tool in nuclear medicine. Here, we attached ^{18}F as radiolabel at different sites of *N*-aryl-oxadiazolyl-propionamides and investigated the affinity, biodistribution and metabolism of these compounds.



Scheme: Different labeling sites at *N*-aryl-oxadiazolyl-propionamides. LG: leaving group

Methods: Compounds were synthesised according to our previously described route (*Rühl et al. Org. Med. Chem. Lett. 2012; 2: 32*). Affinities towards CB2 and CB1 were determined via competitive radioligand binding assays against [^{3}H]-CP55,940. For labelling of **1**, tosylate was selected as LG. Labelling at the aromatic ring in **2** was achieved with trimethylammonium as LG. In vivo organ distribution was investigated on CD-1 mice by injection of 300 - 400 kBq of radiotracer (in 200 µl isotonic saline) in the tail vein. At various times, the activity in the organs of interest was measured via a gamma counter and the percentage of injected dose per gram of wet tissue (% ID/g) determined. Blocking studies were conducted by intraperitoneal pre-injection of 3 mg/kg SR144,528 dissolved in isotonic saline at 10 min prior to the radiotracer. Animals were sacrificed at 60 min p.i., and radioactivity uptake was determined.

Results: **1)** The radiosyntheses were successful in both cases (radiochemical yields ≥ 30%, radiochemical purities ≥ 98%; specific activities ≥ 450 GBq/µmol). **2)** The fluorinated compounds show the same affinity and specificity towards CB2 as the lead compounds. **3)** The radiotracers undergo strong metabolism but **4)** can cross the blood brain barrier (BBB). After five minutes, non-metabolised [^{18}F]**1** and [^{18}F]**2** account for 60% and 2% of the total activity in plasma; 66% and 42% account for non-metabolised [^{18}F]**1** and [^{18}F]**2** in the brain at 30 minutes after injection. The main radiometabolite of [^{18}F]**2** could be identified as the free acid, which has no affinity towards the CB2 receptor.

Conclusions: The introduction of fluorine into different sites of the lead structure does not affect the affinity and the selectivity towards the CB2 receptor. The radiotracers cross the BBB. However, the compounds undergo strong metabolism.

CANNABIS SOCIAL CLUBS AS A NEW SOURCE OF OBSERVATIONAL DATA

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The creation of so-called "cannabis social clubs" in Spain is a social phenomenon that has developed exponentially in recent years. The existence of this form of association allows users continuously access to a source of quality cannabis without having to resort to the black market.

On the other hand, many patients found in cannabis a source of relief for their conditions, and often turn to these associations with the idea of getting a product that meets appropriate quality of production and avoid having to resort to illegal sources.

In the present study we analyze observational data obtained from the work done by a family physician to these kind of patients in different Spanish associations. In all cases the patients received a questionnaire where they provided data such as route of administration, daily amount used, degree of improvement of symptoms and eventual adverse side effects resulting from their self-medication with cannabis, among others.

Methods:

A questionnaire was applied to patients at the first consultation with the doctor. In this questionnaire, the questions were related to the onset of cannabis use, route of administration, the condition for which cannabis is used for medicinal purposes, knowledge of such consumption by other health professionals, level of experience with cannabis prior to onset of the disease and the need for dose modification over treatment.

Results:

As remarkable data we can point that patients use cannabis inhaled in cigarettes (43,07%), oral (20%) or sublingual (15,38%). Most of them use less than 1g per day (62,5%), have informed their doctors about their cannabis consumption (59,45%), have not had to modify the dose in the last three months (71,42%) and use cannabis for diseases involving pain (39,56%) or cancer-related symptoms (16,6%).

Conclusions:

While the patients have no access to other sources of cannabinoids, the use of cannabis in natural way remains a valid option for patients who improve their symptomatology using cannabis in its original form.

ANTIFUNGAL ACTIVITY OF THE VOLATILE OIL OF HIGH POTENCY *CANNABIS SATIVA L.* AGAINST *CRYPTOCOCCUS NEOFORMANS*

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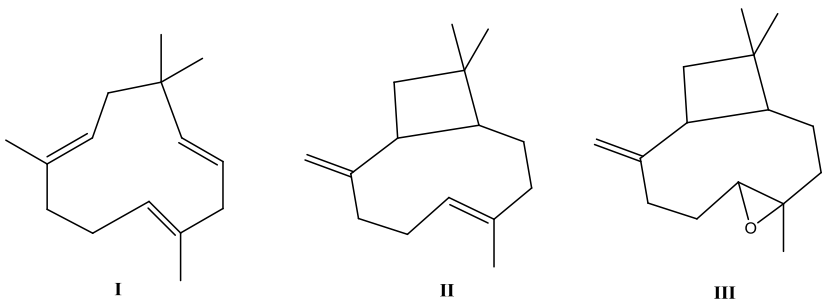
In continuation of our search for bioactive compounds from cannabis, biologically guided fractionation of the volatile oil of high potency *Cannabis sativa* resulted in the isolation and identification of antifungal sesquiterpenes against the human pathogen *Cryptococcus neoformans.*

Methods: Many chromatographic techniques were used for fractionation and isolation of bioactive metabolites from cannabis volatile oil. These techniques include; Vacuum Liquid Chromatography (VLC), Gravity Column Chromato-ghraphy (GCC), Flash Column Chromatography (FCC), Solid Phase Extraction (SPE) and Thin layer Chromatography (TLC). The identification of the isolated compounds was carried out using GC/FID, GC/MS, 1DNMR and 2DNMR

Results: **1)** Seven fractions (A-G) were collected from the VLC and fractions A, C and D displayed good antifungal activities against *Cryptococcus neoformans.* **2)** Seven subfractions were collected from the Si gel column chromatography of the active fraction A. **3)** Three compounds were isolated and their chemical structures were identified as α-caryophyllene (**I**) , β-caryophyllene (**II**) and caryophyllene oxide (**III**). **4)** α-caryophyllene showed good and selective antifungal activity against *Cryptococcus neoformans* with IC_{50} value of 1.18 µg/mL, while, β-caryophyllene showed weak activity (IC_{50} 19.4 µg/mL). **5)** Caryophyllene oxide was inactive.

Conclusions: We conclude from these findings, that **a**) The volatile oil of *cannabis sativa* has potent antifungal activity. **b)** The sesquiterpenes are responsible for the antifungal activity of the volatile oil **c**) α-caryophyllene (**I**) is more active than β-caryophyllene (**II**), while, caryophyllene oxide (**III**) is in inactive **d)**Further investigation is needed for fractions C and D to isolate other active metabolites.



I **II** **III**

DISTINCT ALTERATIONS OF THE ENDOCANNABINOID SYSTEM IN HUMAN MYELOID AND PLASMACYTOID DENDRITIC CELLS DURING MULTIPLE SCLEROSIS

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The immunopathogenesis of multiple sclerosis (MS) has always been thought to be driven by chronically activated and autoreactive Th-1 and Th-17 cells. Recently, also dendritic cells (DC) have been thought to significantly contribute to antigenic spread and to maturation of adaptive immunity, and have been linked with disease progression and exacerbation. Yet, the role of DC in MS pathogenesis remains poorly understood.

Methods: We compared the level of cytokines production by myeloid dendritic cells (mDC) and plasmacytoid dendritic cells (pDC) in healthy subjects and MS patients, following *in vitro* stimulation of TLR7/8. We also evaluated the effect of the main endocannabinoid, anandamide (AEA), in these DC subsets and correlated cytokine levels with defects in the endocannabinoid system (ECS).

Results: **1)** mDC obtained from MS patients produce higher levels of interleukin-12 and interleukin-6, whereas pDC account for lower levels of interferon-α compared to healthy subjects. **2)** AEA significantly inhibited cytokine production from healthy mDC and pDC, as well as their ability to induce Th-1 and Th-17 lineages. **3)** We also found that in MS only pDC lack responsiveness to cytokine inhibition induced by AEA. **4)** Consistently, this specific cell subset expresses higher levels of the anandamide hydrolase FAAH (fatty acid amide hydrolase).

Conclusions: Our data disclose a distinct immunomodulatory effect of AEA in mDC and pDC from MS patients, which may reflect an alteration of the expression of FAAH, thus forming the basis for the rational design of new endocannabinoid-based immunotherapeutics targeting a specific cell subset.

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Acknowledgements

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LICENSED MEDICAL CANNABIS PATIENTS REPORT SAFE DRIVING BEHAVIOR

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Introduction

Israel has been operating a Medical Cannabis Program since 2001. In recent years the number of patients has been raising intensely and is estimated to be over 11,000. The Ministry of Health (MOH) is issuing licenses to patients upon approval of specialist's recommendations. Many of those patients are also holding a driving license and driving a motor vehicle. Moreover, for some paralyzed patients driving is the main way of mobilization. Under the Israeli law, it is illegal to be driving under the influence of cannabis (and other drugs) and Law does not distinguish licensed patients from illegal users. In fact, it is even illegal to be driving if one has any residuals of a dangerous drug in his body.

Research on this field is limited, as the methodology of most studies consider cannabis as an illegal drug and not as medical therapy.

The special legal situation in Israel and the high number of licensed patients allow us to examine the effect of cannabis consumption on driving in an unusual methodological concept. Whereas in most of the research done so far the applicants were volunteers, in this research we interviewed patients who legally and regularly use cannabis in their day-to-day life for symptoms' relief and under medical supervision.

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Method

57 licensed patients were interviewed for this research. For the control group, we surveyed another group of 56 people who do not use medical or recreational cannabis. We used the Hebrew translation of the Driving Behavior Questionnaire (DBQ) to evaluate the behavioral aspects of driving skills and also asked about age, gender and driving experience. For the medical cannabis users group, a few questions were added in order to assess some patterns of use – way of administration, average amount used daily, average times they use cannabis during the day and the time during the day in which they consume the cannabis.

Results

Both descriptive data and statistical tests were processed with IBM SPSS-20 program. Reasons for using the medical cannabis were pain (70%), cancer (12%) and others (18%). Most of the patients (74%) reported using medical cannabis throughout the whole day and the main way of administration was smoking (87%). The mean age of the patients was 40 years and the mean age of the control group – 36. The patients were using cannabis under a license for a mean time of 2.5 year. Mean quantity of cannabis used daily is 2.3 grams. We used an independent T-test to compare the average score of the DBQ. Medical cannabis users have shown an average score lower in 43% than the non-cannabis users (sig. < 0.05). No correlation was found between the amount used daily and the average score on the DBQ.

COMPARATIVE STUDY FOR THE QUANTIFICATION OF THC, CBD AND CBN BETWEEN ALPHA-CAT TLC PROTOCOL AND GC-FID

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Cannabis consumption all over the world is increasing which stress the need for risk prevention and for understand its therapeutical value and potential side effects. The recognized analytics techniques are expensive and need high scientific background that limit the application. Therefore there is a need for an inexpensive techniques to allow large testing procedure in the cannabis industry.

In the industrial hemp there is a need of THC free certification for government officials in order to allow more farmer to cultivate cannabis for its industrial use. As well, the medicinal cannabis industry need cannabinoid labeling for therapeutic guidance and risk prevention

Alpha-CAT is a Cannabinoid Analysis Test KIT using Thin Layer Chromatography (TLC) that enable to identify the 6 main cannabinoids, CBD, CBN, THC, THCV, CBG, CBC. It was develop from Fishedick et al 2010 publication done at Leiden University in the Netherlands.

In alpha-CAT ‘s protocol, the TLC plates were developed using a solvent and colored using B-fast blue dye. For quantification it was used pure cannabinoid reference standards (THC, CBD and CBN). Dilutions range of 1 from 1% to 20% were prepared and spotted on silica G25 TLC plates. Quantification was achieved by Photoshop and excel computer program using ratio of pixel counting/surface area. It was obtained a regression curve which showed a linear correlation. From this regression line THC, CBD and CBN calibration charts were designed to have a handy and visual way to measure % according to spot size showed on the revealing plates.

A comparative study was done between Fundación CANNA (FC) using Gas Chromatography - Flame Ionization Detector (GC-FID) and alpha-CAT kit for the quantification of THC, CBD and CBN.

Methods: 4 homogenized batches of different cannabis varieties were used for the test. 3 samples of each batch were analyzed by TLC and 3 by GC/FID. The laboratory procedures were done at FC location and the TLC results were blind tested by sending plate image scan to alpha-CAT laboratory in Marseille for cannabinoid quantification using alpha-CAT calibration chart.

Results: The average percentages of each samples were compared. Differences between 15,40% and 48,82% were detected in quantifying THC and a 34,25% in CBD when comparing TLC method for testing with GC-FID. The ratio of THC:CBD was proportionally comparable. For CBD below 1% and CBN there was an incoherent in terms of quantification even though they were detected properly,.

Conclusions: This is the first straight forward comparative study between GC-FID and the TLC alpha-CAT's protocol. Alpha-CAT's testing method has shown to be a valid qualitative tool for cannabinoid detection and gives reproducible results. However, only technicians well trained with alpha-CAT's protocol can obtain semi quantitative results by using the alpha-CAT’s cannabinoid calibration charts. Gas/Liquid chromatographic analysis need to be used to quantify precisely cannabinoids, and even other important secondary active compounds present in Cannabis..

HEMP MERISTEM-MACERATES IN GEMMOTHERAPY

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Vital processes in buds and shoots ensure the mobilisation of lipids as fatty acids as well as the digestion of storage starch into diverse sugars. There are high levels of oligosaccharides, phytohormones (e.g. auxines, gibberellines), aminoacids or proteins and enzymes. All these building materials are needed for the metabolism in division-active tissue (meristemes). Moreover primary substances are available for the synthesis of species-typical secondary plant compounds.

Phytotherapy knows as starting material the whole plant, roots, leaves, stalks, flowers, seeds or even barks. In gemmotherapy, as a specialised form of phytotherapy, there are macerates used from meristemes of buds and shoots. The total information, the vital and growth force of the bud is utilized as regeneration and healing power for humans.

Therapy-specific or as a preventive measure gemmotherapeutics take an increasingly important role in natural medicines. With its vasodilatative and catabolic effect they help release the body tissue from endotoxins and ensure a better regeneration and healing. With the direct protein communication in the cells gemmotherapeutics stimulate the immune system and arrange for the re-establishment of the protein balance in the blood.

In this context, for research and development, the Terra Energetika GmbH, as first company worldwide, is focussing on the manufacture and application of meristem-macerates from Cannabis sativa L.

Method: Cell tissue from diverse, originally continental landraces of *Cannabis sativa* L. (Tattwas certified bio-dynamic hemp cultivation) was used as starting material. This material was further processed according to recipes of the Terra Energetika GmbH. From the single buds and shoots, carefully picked by hand, a macerate of ethanol, glycerine and water was manufactured under good manufacturing practice. After the maturing time the extract was filtered and scientifically investigated for dissolved ingredients, purity and legal conformity. The scientifical and analytical examination in compliance with GMP and ISO 17025 has been carried out by an international renowned analytic laboratory and by our partner laboratory Ai Lab Swiss (plant analysis) department of the Ai Fame GmbH.

Result: Among the principal ingredients a number of important cannabinoids were found for example cannabichromen (CBC) or cannabigerol (CBG) as well as other ingredients such as α-bisabolol and vitamin E acetate.

Conclusion: These ingredients show a broad application area and are proved in case of infections, inflammations, pain, psychical discomforts (e.g. depression, stress, fear etc.), loss of appetite, psychosomatic disorders, skin diseases and asthma. For example α-bisabolol that was originally isolated from chamomile oil is not toxic for healthy cells but induces apoptosis (natural cell death) in cancer cells.

The gemmotherapeutic from summer lime (*Tilia platyphyllos* Scop.) is known for its anxiety reducing, relaxing and nervine quality and is successfully applied in states of fear as well as for gastritis and bulimia. For depressive states fig tree (*Ficus carica* L.) as well as weeping birch (*Betula pendula* Roth) strengthens nerves and with both combined it could be achieved an even better success.

Initial studies have shown, that concerning ingredients of the hemp meristem-macerate there are a lot of possible applications that have considerable importance for medicinal and therapeutical approaches.

NEUROPSYCHATRIC USES OF MEDICAL CANNABIS/MARIJUANA: FOCUS ON POST-TRAUMATIC STRESS DISORDER

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Background: The current medical treatment of the neuropsychiatric disorders, such as Multiple Sclerosis, Epilepsy, Parkinsons's disease, Tourette syndrome, Posttraumatic stress disorder (PTSD) is unsatisfactory, since it does not provide sufficient coverage of symptoms and usually cause multiple adverse and common bothering side effects. This is the main reason for poor quality of life (QoL) of the patients and frequently lead them to therapy neglect and inadherence.

In the recent years we observed the rapid growing of the clinical experience (case reports/series) and studies (naturalistical/observational, open and even controlled) with Cannabis/marijuana in patients with different neuropsychiatric disorders, such as Parkinsons's disease, Tourette syndrome, Posttraumatic stress disorder (PTSD) etc. Most reports are describing the combine approach, where the Cannabis/marijuana is given as Add-on therapy to the previous medical regimen, under the continuous follow-up. The authors providing the data showing an improvement not only in particular neuropsychiatric symptoms, such as tremor, seizures, spasticity, tics and hypervigilance, but also in reduction of side effects. The usage of additional medications, such as sedatives and tranquilizers is going down. As a result: an improving in overall QoL and in better treatment adherence and therapeutic and rehabilitative cooperation.

The primer: The example, where the large group of PTSD patients is followed up longitudinally and specific positive change were registered, using the standardized instrument, in measures of traumatic symptoms, pain perception, Quality of Life and in Clinical Global Impression, is provided.

Discussion: However, despite of all information existing and readily available (in Web, and special sites), the majority of the physicians (here: neurologists and psychiatrists) are still reluctant to such approach, and are not ready even to consider a Cannabis/marijuana to be a part of the management of neuropsychiatric symptoms in these patients. Misleadingly, they are sure, that usage of medical Cannabis/marijuana in these patients will harm inevitable their health and social status, and lead them to become a heavy drug addicts.

In order to contradict these myths and prejustice, concerning the Medical Cannabis/marijuana, and to provide our colleagues an appropriate, objective and balanced information, educational programs and other activities should be elaborated and introduced, with the special emphasize on the efficacy and safety issues of the Medical Cannabis/marijuana usage in patients with different neuropsychiatric disorders.

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REDUCTION IN METHADONE CONSUMPTION AND WITHDRAWAL SYMPTOMS WITH INGESTION OF STANDARDIZED ORAL CANNABIS. AN OBSERVATIONAL/FEASIBILITY STUDY.

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A short (3 month) study was undertaken to determine the feasibility of a larger placebo controlled, double-blind clinical trial regarding the efficacy and safety of standardized oral cannabis in reducing methadone consumption and withdrawal.

Methods: A study population was selected from the membership of Eden Medicinal Society a non-profit organization located in the downtown Eastside area of Vancouver, British Columbia, Canada, a part of the city notorious for a high rate of drug addiction, HIV and Hepatitis C infection. Selection criteria was based on members that responded to an advertisement posted in the Society for those with long term methadone use to take part in a study. Of the members that came forward, further selection was based on a general questionnaire regarding drug history, illness, compliance probability, and willingness to take part. Study participants were tracked over the three-month period with questionnaires, pain charts and regular interviews. Methadone prescription records were monitored to validate claims of methadone use.

Results: Compliance to the cannabis capsules was predicted as a major issue that would make the study not feasible. However, methadone, often described as "liquid handcuffs", by the user, begs compliance to the methadone clinic where daily doses are administered, making a daily trip to the dispensary, possible for many. Of the eleven members that began the study, nine completed and of those all showed a decrease in methadone consumption. The average age of the study participants was 43 years, all but 1 was male. Average years of methadone use was 12.2 (106.7 mg/day) and for cannabis years of use was 27.1 (6.5 grams/day). Overall compliance to the cannabis capsules was 48%, with the highest being 85%, thus making it necessary to have members take capsules home, where a difference was not observed in study parameters (questionnaires, pain charts, prescription records). The average number of capsules required per day was 7, equaling 280 mg of THC as cannabis per day.

Conclusions: Our purpose was to investigate the feasibility of a study to determine the efficacy of standardized, natural product cannabis, as an oral preparation in reducing the symptoms of methadone withdrawal. Taking the evidence beyond the ever mounting anecdotal to one of systematic study leads us to a new starting point. We conclude from this investigation that indeed a reduction in methadone consumption is observed with an intervention of standardized oral cannabis, that the first 50% reduction, is the 'easy' part, the second 50% , being more difficult. In addition it was noted that the more successful members in reducing methadone were those that continued good rapport with the study assistants, indicating an important psychological factor in methadone withdrawal.

MEDICINAL CANNABIS: *IN VITRO* VALIDATION OF VAPORIZERS FOR THE SMOKE-FREE INHALATION OF CANNABINOIDS

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The interest in the medical use of *Cannabis sativa* L. has increased over the past years because cannabinoids have proven to be effective to alleviate and treat various symptoms such as spasticity, pain, nausea, anorexia and depression in a variety of diseases. Main indications for the use of cannabinoids are multiple sclerosis, amyotrophic lateral sclerosis, chronic pain and cancer. Cannabis contains more than 80 different cannabinoids, among which Δ^9-tetrahydrocannabinol (THC) is the primary psychoactive constituent. However, growing evidence has shown that in addition cannabidiol (CBD) has important pharmacological activity that contributes significantly to the clinical effects of Cannabis. Since THC and CBD occur in the plant as their biologically inactive acids (THCA-A and CBD-A) temperatures above 200 °C are needed to deliver neutral THC and CBD. Therefore, Cannabis is usually smoked in combination with tobacco. An alternative is the use of vaporizers, which release THC and CBD into the gas phase without pyrolysis of the plant material, avoiding therefore the formation of harmful combustion products. The aim of this study was to assess the suitability of the four commercially available vaporizers Volcano and Plenty (Storz & Bickel, Tuttlingen, Germany), Arizer Solo (Arizer Tech, Waterloo, Canada) and DaVinci (Organicix LLA, Las Vegas, USA) for the inhalation of cannabinoids from *Cannabis sativa* L. for medical purposes. Consequently, an *in vitro* validation of four vaporizers was performed.

Methods: Two *Cannabis sativa* L. varieties were used (50 mg), one with 4.6 % of total THC (THC type; 90.4 % as THCA-A) and the other with 2.6 % of total CBD (CBD type; 85.8 % as CBD-A). In addition, pure THC and CBD standards in ethanol (2 mg) were vaporized using the same experimental design. The temperature of the vaporizers was set to 210 °C. The vapor was aspirated through a SPE cartridge (Li Chroprep RP-18) and eluted with methanol-chloroform 9:1 (v/v). The mouthpiece, heating chamber and connection tube were rinsed with the same solvent and the fractions collected. The samples were evaporated to dryness under N_2, reconstituted in methanol-chloroform 9:1 and diluted with methanol. The residue was extracted with the same solvent. GC/MS was used to quantitate THC, CBD and CBN (decomposition product of THC) with deuterated internal standards on a DB-1ms column (30 m x 0.25 mm i.d., 0.25-µm film). THCA-A and CBD-A were determined by HPLC-PDA on a Spherisorb ODS I column (125 x 4 mm i.d., 3 µm particle size).

Results: The four devices efficiently decarboxylate acidic THC and CBD (> 98 %) and release neutral THC and CBD from Cannabis into the vapor (54 – 80 % and 51 – 74 %, respectively). The recoveries are similar to those obtained for the standards with 41.6 – 71.1 % and 53.6 – 84.3 % for THC and CBD, respectively. Only trace amounts of THCA-A and CBD-A were found in the vapor, proving that adequate amounts of active THC and CBD are available to the patient. Less than 15 % of the total cannabinoids remain in the residual plant material using the Solo and the Volcano, whereas the release into the vapor is complete with the two other devices (remaining cannabinoids < 5 %).

Conclusions: The four temperature controlled vaporizers revealed efficient decarboxylation of the biologically inactive THCA-A and CBD-A, releasing active neutral THC and CBD into the vapor. Non-pyrolytic inhalation of Cannabis by vaporizers might therefore be a promising alternative to the oral administration of cannabinoids for medical use.

DEVELOPMENT OF NEUTRAL-DAY CANNABIS PLANTS WITH HIGH CBD CONTENT

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Introduction

Cannabidiol (CBD) has proven to posses several therapeutic applications and actually is incorporated in some pharmacopoeias. CBD is naturally found only in plants of the Cannabis genus, from which it is commonly extracted and then purified for pharmaceutical applications. Although in the hemps varieties grown for industrial applications the CBD content prevails on the content of Delta-9-tetrahydrocannabinol (THC), its concentration is still very small (normally around 1,5-3%) and for this reason it would be desirable to use raw material derived from plants
having higher concentrations of CBD.
In the case of short-day cannabis plants, clones from a "mother plant" selected for its high concentration in CBD can be grown in greenhouses all year long under artificial light. Such plants can be also grown in greenhouses or outdoors by using exclusively natural light but only one crop per year can be harvested in those countries, which are far away from the equator, without having to artificially modify the natural photoperiod.
Neutral-day plants start to flower when they reach a certain age, regardless of the photoperiod. The cultivation of neutral-day cannabis plants, usually requiring only 60-80 days from sprouting to harvest, allows several harvests per year using sunlight, which would save energy compared to supply artificial light.
Being not possible to prolong the vegetative state of a neutral-day plant for the purpouse of obtaining clones, in order to identify and select those individuals with high CBD content, it is necessary to analyse the plants in a vegetative state before they start to flower.

Methods: Using the technique of sex reversion by applying STS (silver thiosulfate), females neutral-day plants with pure THC chemotype were fertilized with pollen derived from a sex reversed, female short-day plant with pure CBD chemotype previously selected for its high content on this cannabinoid. From this F1 two individuals were selected to produce the F2 generation of seeds. Progenies derived from F2 plants were grown at 20 hours of artificial light/day in order to detect the neutral-day plants and, by analysing the leaves of the plants in vegetative state by GC/FID, those having a pure CBD chemotype and a neutral-day flowering were crossed resulting in the F3 generation.

Results: All the plants in the F1 were short-day plants and with mixed THC:CBD chemotype. In the F2, a 68% of plants presented a mixed THC:CBD chemotype and a 15% the neutral-day flowering. All the plants in the F3 were neutral-day plants and with a pure CBD chemotype.

Conclusions: The CBD and THC chemotype of plants in the vegetative state is coincident with the chemotype in flowering, so by analysing the leaves by GC / FID is possible to identify and select the plants with the desired chemotype even if the plants have not achieved the maturity. Using the technique described has been produced a generation of plants in which all individuals are females neutral-day plants of CBD chemotype with an average of CBD concentration of 15% and a peak of of 16,69%. The THC concentration was always less than 1%.

RATS CHRONICALLY TREATED WITH A CANNABINOID ANTAGONIST: CONVULSIVE SEIZURES AND DECREASED SYNCHRONIZATION IN THE EEG

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Cannabinoid CB1 antagonists have been widely investigated for possible treatment of obesity, metabolic syndrome and drug addiction. However, in healthy rats chronically treated with an CB1 antagonist muscle spasms have been observed, a severe side effect. The present study was performed to investigate the origin of these spasms. Rats (n=200) were treated with a coded CB1 receptor antagonist or with vehicle. During a six month pharmacological study, it was found that severe muscle contractions indeed developed; in 15% of animals treated with a low dose of the drug (1; 1 mg/(kg.day) for male; female rats) and in up to 70% of animals from the highest dose groups (3; 2 mg/(kg.day) for male; female rats).
EEG and video recordings were made from 37 animals selected from the original group of 200. During a 24 hour recording period, 26% of animals treated with this CB1 antagonist were found to have between 1 and 21 convulsive seizures in the EEG, whereas controls remained seizure free. Moreover, all observed spasms were found to be coincided by seizures in the EEG and vice versa, indicating that the spasms were not merely peripheral muscle contractions but were induced centrally.
Analysis of the ongoing EEG using synchronization likelihood (SL), demonstrated that SL between activity of the cortex, the thalamus and the hippocampus in the theta band was significantly lower for all treated animals compared to the controls. Also a shift in theta peak frequency was found.
This study confirms our previous observation that long term blockade of the endogenous cannabinoid system can induce epileptic seizures (1). Moreover it suggests that the effects of blocking the on-demand protection the endocannabinoid system provides against the consequences of a variety of injuries goes beyond the scope of seizures.

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A CANNABIS-DNA ANALYSIS METHOD TO SECURE THE GENETIC IDENTITY OF CANNABIS PLANTS

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A Cannabis-DNA analysis method based upon PCR of short tandem repeats (STRs) was developed to analyse the genetic fingerprint of Cannabis plants, i.e. distinguish between apparently morphologically or chemotypically identical plants. This analysis method is fast and reliable and can even discriminate between two closely related individuals (sibblings).
In this presentation we are going to show some typical examples and detail the genetic background for a better understanding.
The Cannabis-DNA analysis method can be used as a quality application to permanently check the genetic identity of cloned plants or to check and control the breeding success in plants of pharmacological or agricultural interest.

EPILEPTOGENESIS UNDER CONTROL OF THE ENDOGENOUS CANNABINOID SYSTEM?

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The endocannabinoid system is suggested to provide endogenous protection against seizures. Using a rodent model of audiogenic epilepsy, we explored whether activation of CB1 receptors is involved in this type of epilepsy. In study 1 we studied the influence of a CB1 agonist (WIN55,212-2) on the process of epileptogenesis, i.e. the kindling process [1]. In study 2 we studied a CB1 antagonist (rimonabant) on the reverse process, i.e. on the phenomenon of acquired resistance to seizures [2].
In study 1, the CB1 agonist study, we used Krushinsky-Molodkina (KM) rats with genetic audiogenic epilepsy. In these animals, repeated induction of audiogenic seizures results in a progressive prolongation of the seizures. This phenomenon, known as audiogenic kindling, is due to generalization of the seizures: from the brainstem to the forebrain. Administration of a single dose of 4 mg/kg of WIN55,212-2 one hour before the 4th induction of an audiogenic seizure delayed the kindling process with two weeks. [1]
In study 2, the CB1 antagonist study, Wistar rats were selected on susceptibility to audiogenic seizures. Three groups were selected: 1) non-epileptic rats, 2) rats with acquired resistance to audiogenic seizures and 3) rats with reproducible audiogenic running seizures. Subchronic treatment with rimonabant (5 daily dosings of 30mg/kg) had no effect in the group of non-epileptic rats (for chronic treatment see ref [3] and the abstract by Perescis et al.). In the second group of rats this subchronic treatment reverted the acquired seizure resistance: the audiogenic running seizures reappeared after the end of treatment. In the third group of rats, with reproducible running seizures, rimonabant induced *de novo* a limbic component that is also seen during audiogenic kindling and therefore indicates propagation of the brainstem seizures to the limbic forebrain. [2]
These results support the idea that the cannabinoid system is an essential part of the endogenous anticonvulsive mechanisms of the brain. Moreover, the results suggest that potentiation of the endogenous cannabinoid system might mitigate the epileptogenic disease process in patients with a progressive course of epilepsy.

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THE EFFECT OF Δ^9-TETRAHYDROCANNABIVARIN ON FOOD DEPRIVATION-INDUCED FOOD INTAKE AND UPPER GASTROINTESTINAL MOTILITY: DIFFERENCES FROM RIMONABANT

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Δ^9-tetrahydrocannabivarin (THCV), the propyl homologue of Δ^9-tetrahydrocannabinol (THC) in *Cannabis*, was recently rediscovered as a potentially therapeutically useful cannabinoid due to its properties as a neutral CB1 antagonist and CB2 partial agonist (see Pertwee, *Br J Pharmacol* 153:199-215, 2008, for review). THCV, as previously reported for CB_1 inverse agonists, such as AM251 and rimonabant, was shown to inhibit *ad libitum* food intake in lean mice (Riedel et al., *Br J Pharmacol* 156:1154-66, 2009), but not in *ob/ob* obese mice or mice with diet-induced obesity (Wargent et al., *Nutr Diabetes*, 3:e68,2013). In these latter mice, higher hypothalamic endocannabinoid levels than in lean mice are found, and CB1 inverse agonists are instead even more efficacious at inhibiting feeding (Di Marzo et al., *Nature*, 410:822-5, 2001; Cristino et al., *Proc Natl Acad Sci USA*, 110:E2229-382013). Since also in food-deprived animals, elevated hypothalamic endocannabinoid levels have been measured (Kirkham et al., *Br J Pharmacol*, 136:550-7, 2012), we have examined here if THCV, in comparison with rimonabant, reduces food deprivation-induced refeeding in mice. Furthermore, we have assessed the effect of both THCV and rimonabant in another typical in vivo assay of CB_1 inverse agonist/antagonist activity, that is, the stimulation of upper gastrointestinal transit in mice.

The effect of increasing doses of THCV (3-40 mg/kg, i.p.), AM251 or rimonabant (10 mg/kg, i.p.), or vehicle (2.5% ethanol in sesame seed oil), was evaluated in mice fasted overnight (15h) from 18.00h to 9.00h. The day after fasting, mice were placed in individual cages without food. At 10.00h the mice were dosed, food was given 30 min later and the amount eaten measured at 1h, 2h and 3h. Upper gastrointestinal transit was measured by using the charcoal method, consisting of the measurement of the distance travelled by a charcoal marker, expressed as a percentage of the total length of the small intestine from pylorus to caecum. THCV (1-20 mg/kg), rimonabant (0.1-5 mg/kg), the CB_1/CB_2 agonist WIN55,212 (0.1-5 mg/kg), or combinations of WIN55,212 (1 mg/kg) or THCV (5 mg/kg) with a per se inactive dose of rimonabant (0.3 mg/kg) (all dissolved in DMSO), were administered i.p. 20 min before charcoal administration. Transit was measured 20 min after charcoal administration.

Unlike AM251 or rimonabant, which caused a strong inhibitory effect of food deprivation-induced refeeding, THCV did not produce any effect on feeding up to the highest dose tested (40 mg/kg). Like WIN55,212, which dose-dependently inhibited gastrointestinal transit, and unlike rimonabant, which instead caused a dose-dependent elevation of gastrointestinal transit, THCV, starting from 5 mg/kg, inhibited transit. This action, unlike the similar effect of WIN55,212, was not reversed by a per se inactive dose of rimonabant.

We show that THCV does not behave like rimonabant in two typical in vivo models of CB_1 inverse agonist/antagonist activity. In particular, THCV seems to lose its capability of inhibiting food intake in the presence of a higher hypothalamic endocannabinoid tone, such as during obesity or food deprivation-induced feeding behavior, which argues against a CB_1 inverse antagonist activity in this context, whereas it shows a non CB_1-mediated inhibitory effect on upper gastrointestinal transit.

PLANT REGENERATION OF *CANNABIS SATIVA* [L.], THROUGH ANTHER CULTURE (in vitro) OF THE CULTIVAR "USO' S"

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Cannabis sativa [L.] – hemp - is an important plant in medicine and pharmacy. The unequal concentration of specific cannabinoids in different cultivars, lead to the fact, that a collection of valuable plants with medical compound (high CBD, high CBG, etc.) should be established. A university could be the right place to provide proven medical plant material worldwide. There are not many studies on tissue (*in vitro*) culture of hemp. Various scientists reported, that callus readily produced roots, but was unreceptive to shoot formation. The goal of the study was, to identify PGRs (plant growth regulators), that enable a plant regeneration out of certain selected tissue. We have tested various PGRs in different concentrations and combinations. This study describes the standardization of an efficient *in vitro* propagation and hardening procedure for obtaining plantlets from male flowers (anthers) of *Cannabis.*

Methods: All the process is divided into five stages: **1.)** Culture-start, **2.)** Regeneration: Single flowers of male C. sativa plants has been exposed, to four media all half strength Murashige and Skoog medium supplemented with 20 g/l sucrose and 7 g/l agar at a pH of 5.8 and under dark condition. The different PGR mixes **(a-d)** has been: **a.)** 2 mg/l BAP+ 0.025 mg/l IAA; **b.)** 2.0 mg/l BAP + 0.1 mg/l TDZ + 0.025 mg/l IAA; **c.)** 0.5 mg/l TDZ + 0.025 mg/l IAA; **d.)** 2 mg/l MT + 0.025 mg/l IAA. However, the explants were remaining about 40 days on the medium. After that the stage **3.)**, vegetative shoot growth on full MS-medium supplemented with 20 g/l sucrose, 7g/l agar and 0.1 mg/l TDZ, follows for 28 days under Fluorescent light. The next step **4.)**, rooting, was induced on 1/3 MS-medium containing 0.5 mg/l IBA and was requiring about 28 days. Step **5.)**, the final stage of acclimatization was done in a greenhouse with high level of relative humidity; it took 3-4 weeks.

Results: Regeneration of plantlets from *C. sativa* can be obtained by male flower buds (anther) culture. All the process is taking about 120 days, for an in vitro culture this is fast. The best proliferation was on MS-medium **b.)** supplemented with 20.0 g/l sugar, 7.0 g/l agar, 2.0 mg/l BAP, 0.1 mg/l TDZ and 0.025 mg/l IAA. A development of adventitious shoots could be observed in low frequency. Nevertheless, the successful rooted plantlets could be transferred into pots filled with soil (*ex vitro*); the plants had shown regular growth morphology. Astonishing, the small plant (regenerated from a male flower) was showing after a generative stage of additional 4 weeks, female flowers. Medium **a, c** and **d** were not able to initiate plant regeneration or even callus formation.

Conclusion: We conclude from these findings, that the presented *in vitro* system is suitable for *C. sativa* propagation and storage of high valuable elite cultivars (medical or industrial purpose). It could be used for biotechnology, breeding processes (creation of haploid plants), pharmaceutical industry, medical facilities, change of sex in individual plants and so on. Besides that, the male flower buds (anther), seems to be an excellent source of tissue for regeneration processes.

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